Projet Biosystémique

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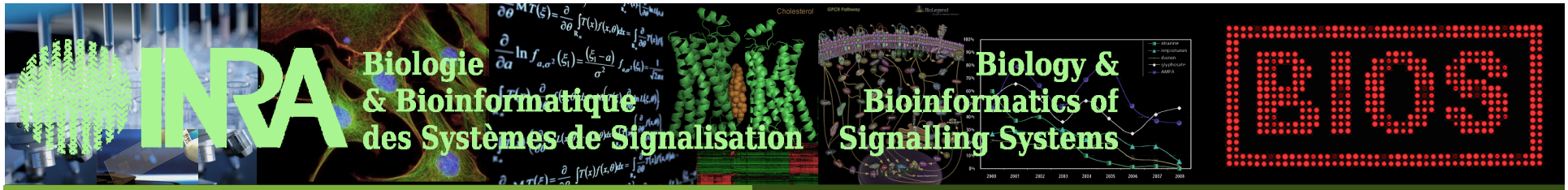
Université de Tours



Introduction

« Objet biologique » : les voies de signalisation dépendant des récepteurs couplés aux protéines G (GPCR).

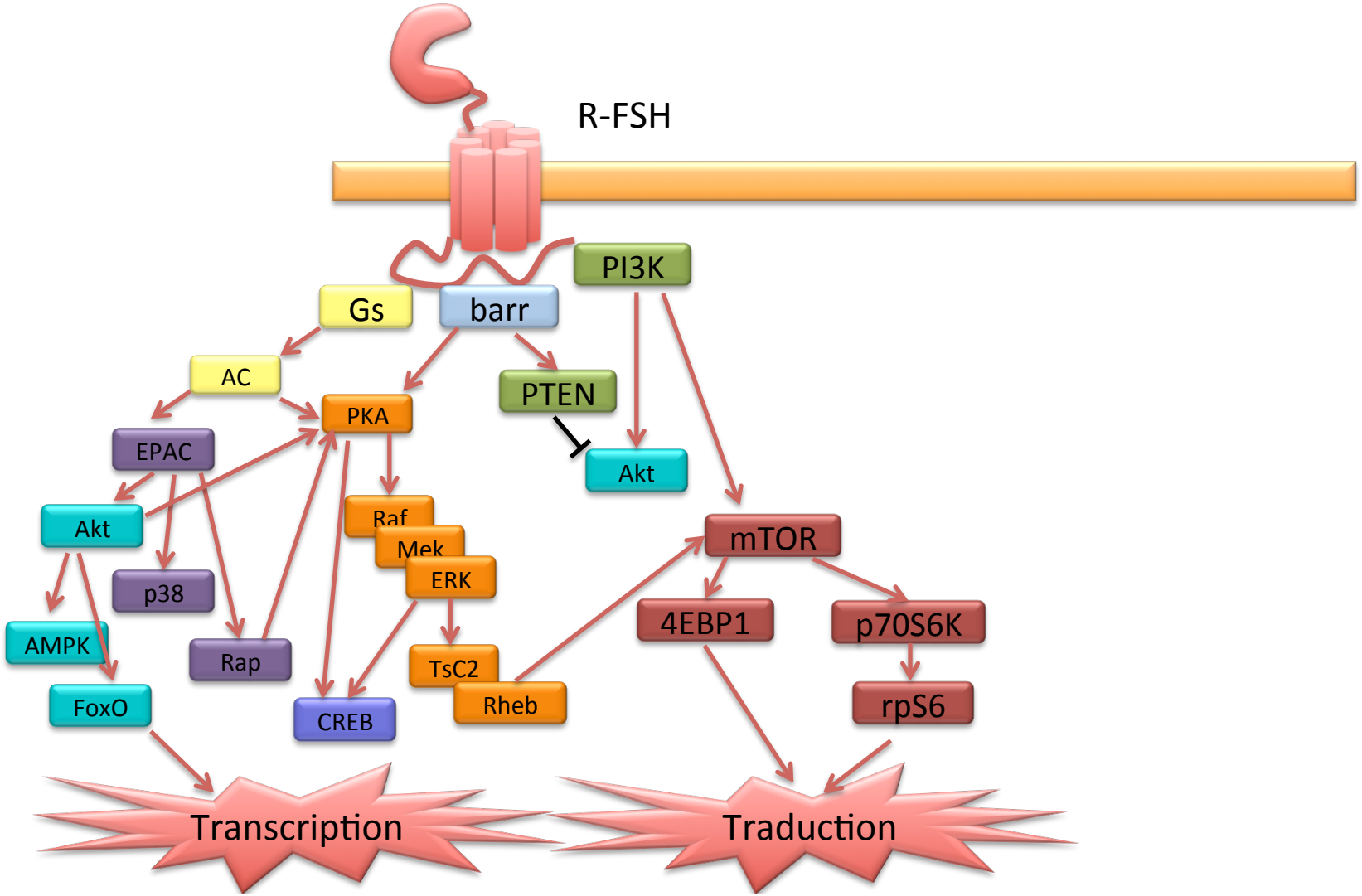
- Les GPCR sont des protéines situées dans la membrane des cellules.
- Ces molécules sont des récepteurs, elles se lient de manière spécifique à un ligand : hormone, ions, lipides, etc.
- La liaison au ligand déclenche un grand nombre de réactions à l'intérieur de la cellule : le réseau de signalisation. Ceci permet à la cellule de réagir au signal que représente le ligand.
- Les GPCR sont très spécifiques et membranaires => cibles idéales pour les médicaments. Presque la moitié des médicaments sur le marché.

Mais :

- Seulement 15% des récepteurs « utilisés » par la pharmacopée
- Effets secondaire

=> nécessité de bien connaître le réseau de signalisation

Introduction



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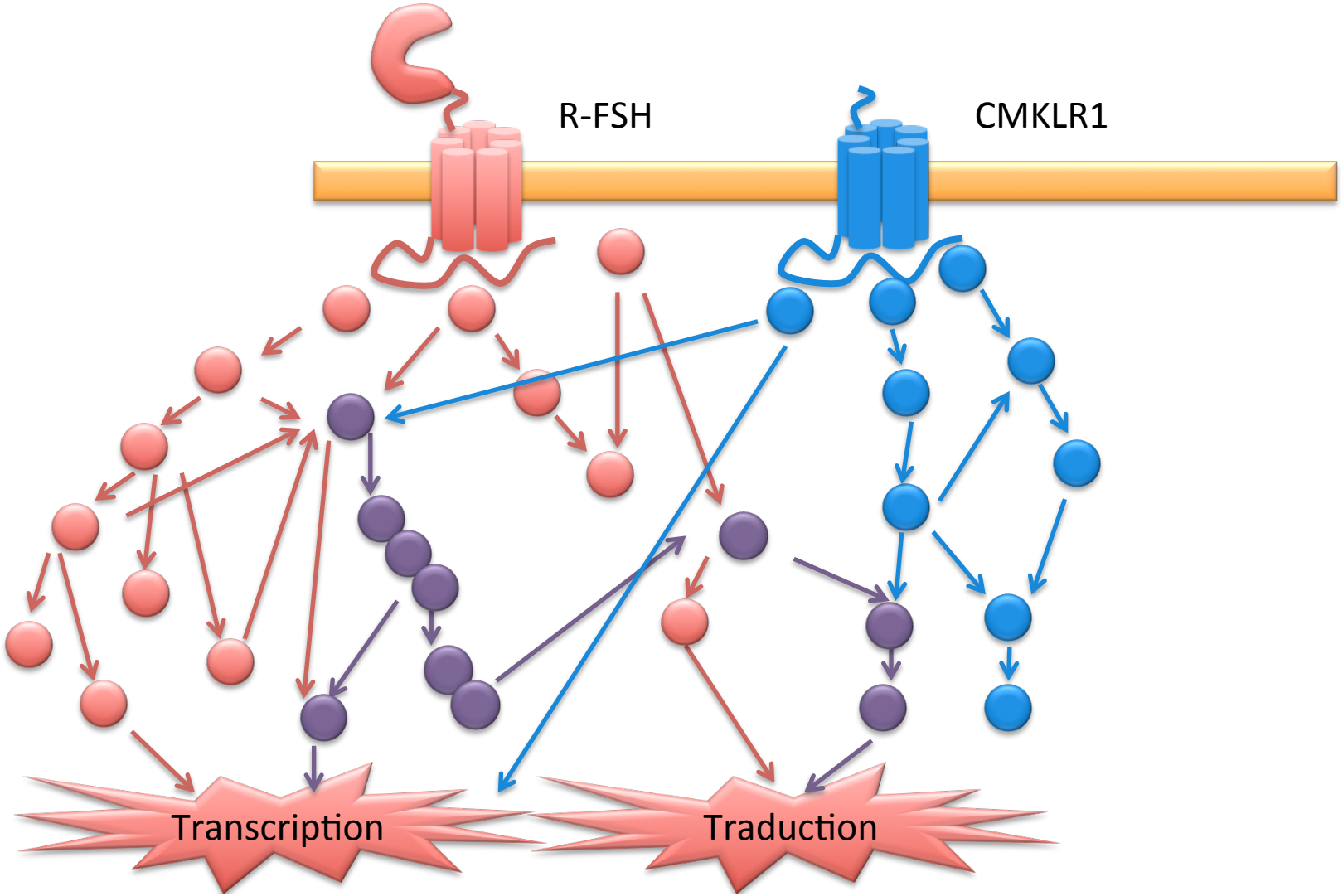
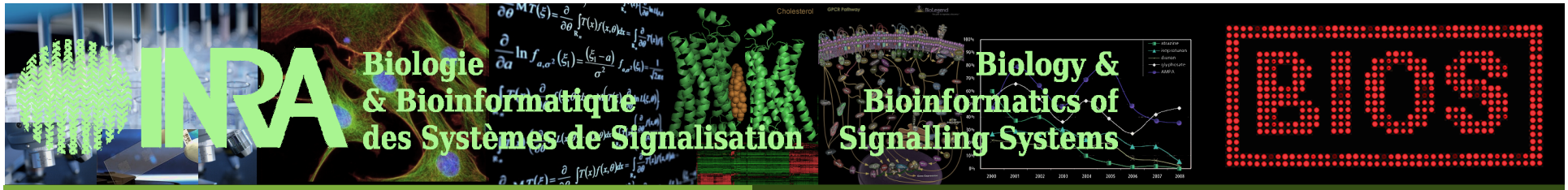




Introduction

Construire le réseau :

- Trouver les expériences dans la littérature
- Les assembler pour former le réseau

Mais :

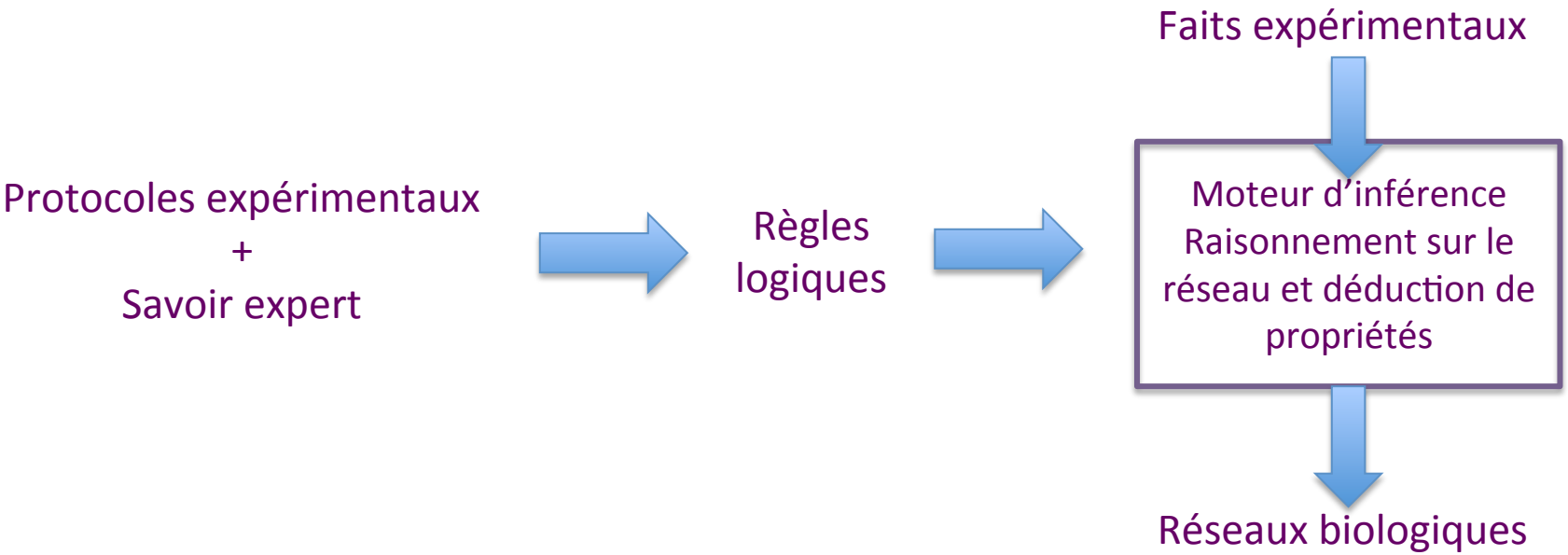
- la littérature concernant les voies de signalisation est énorme (1993 articles pour le seul mois de septembre 2015) ;
- les résultats expérimentaux sont souvent sur- ou sous-interprétés par les auteurs ;
- chaque article ne contient qu'un petite partie des données nécessaire à la reconstruction du réseau complet.

⇒ Il faut automatiser !

Développement d'une méthode d'inférence automatique.
On ne prend en compte que les résultats des expériences, et pas leur interprétation.

Inférence automatique

Principe de la méthode



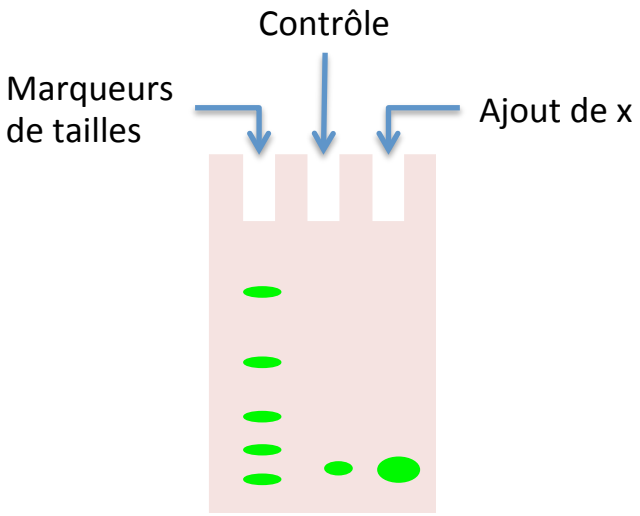
Inférence automatique

Règle

SI condition(s) ALORS conclusion(s)

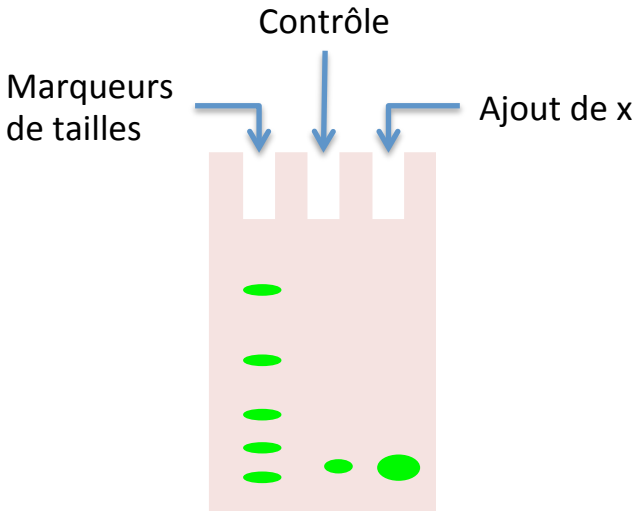
- Simples : conclusion(s) obtenue(s) à partir d'une expérience
- Complexes : conclusion(s) obtenue(s) à partir de la combinaison de conclusions précédentes

Phosphorylation Assay



On observe plus de y phosphorylée en présence de x

Phosphorylation Assay



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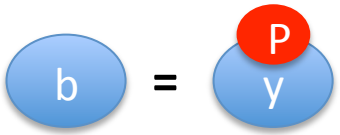
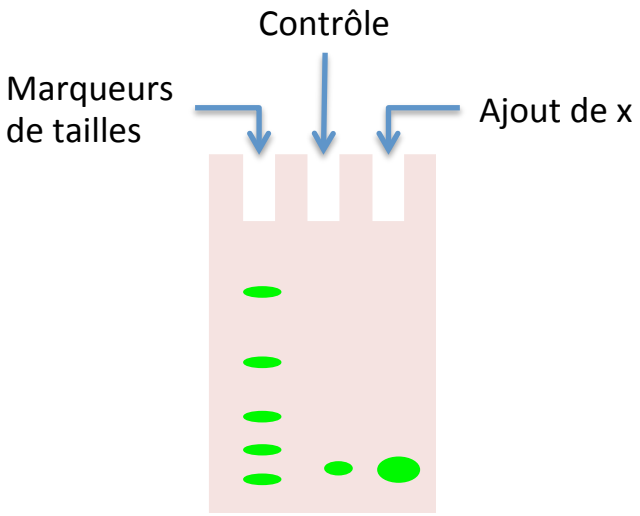


b est la forme phosphorylée de y

Phosphorylation Assay



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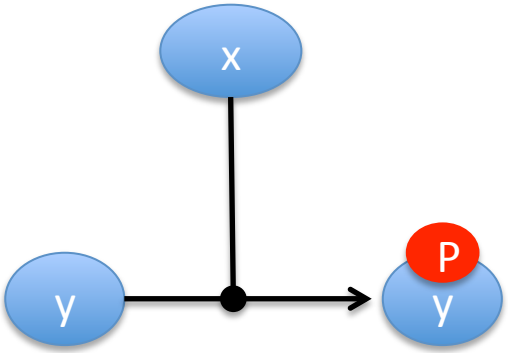
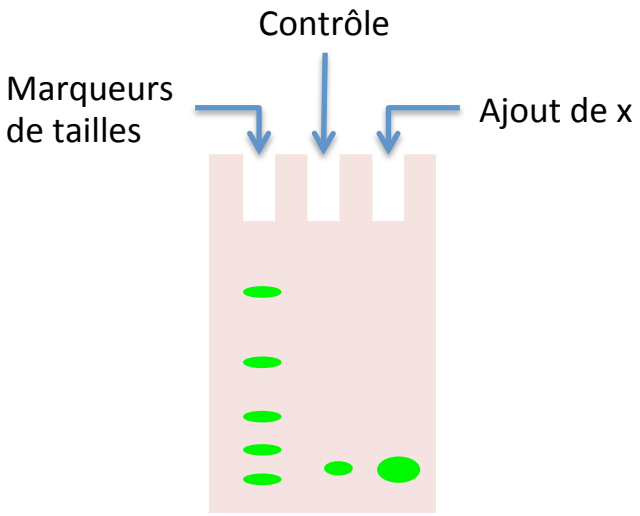


x active la phosphorylation de y

Phosphorylation Assay



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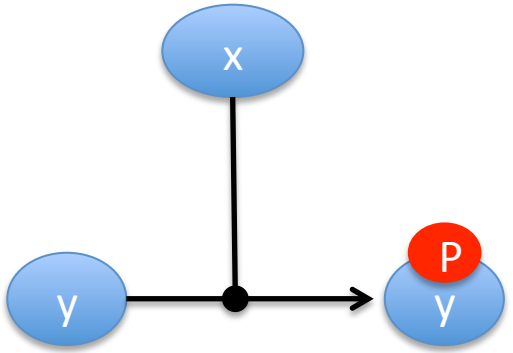
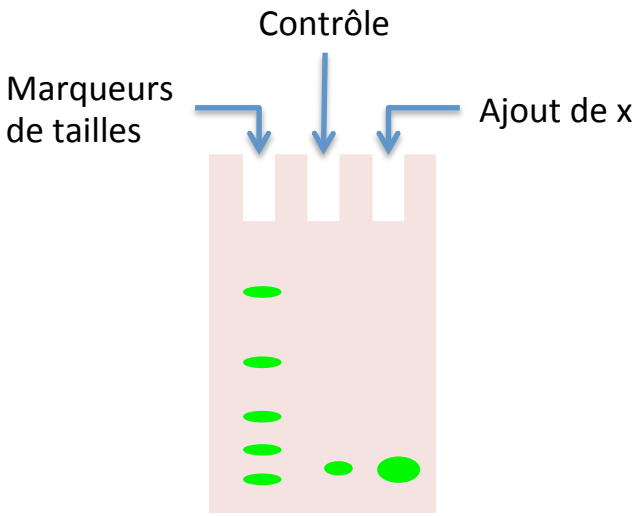
PA(x;y;py;increase)



x active la phosphorylation de y

PHOSPHORYLATE(x; y; py; increase)

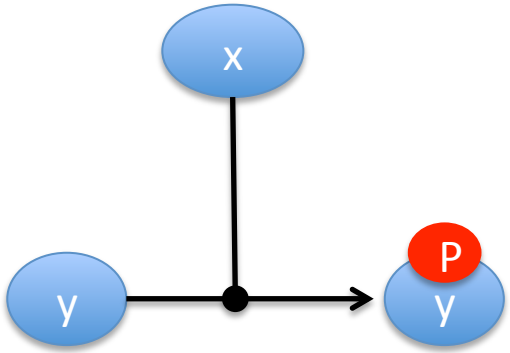
Phosphorylation Assay



On observe plus de y phosphorylée en présence de x

PA(x;y;py;increase)

IF PA(x;y;b;increase)
THEN PHOSPHORYLATE(x;y;py;increase)

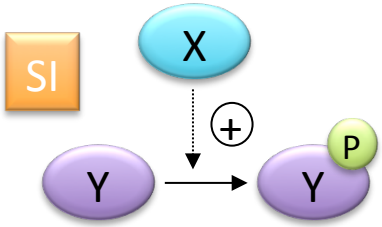


x active la phosphorylation de y

PHOSPHORYLATE(x; y; py; increase)

Règles secondaires

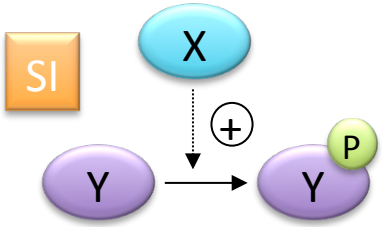
Combiner les conclusions



On sait déjà que phosphoryle y

Règles secondaires

Combiner les conclusions



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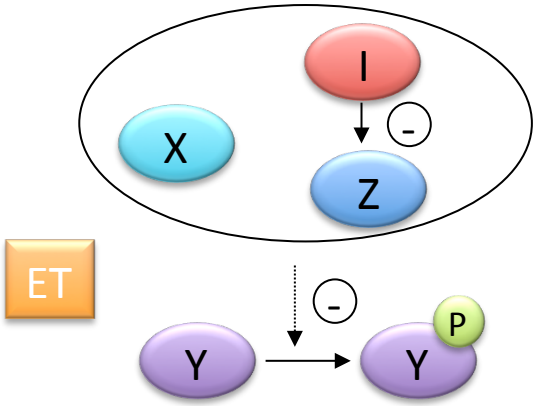

On ajoute un inhibiteur de la protéine z, on observe que la quantité de py diminue

Règles secondaires

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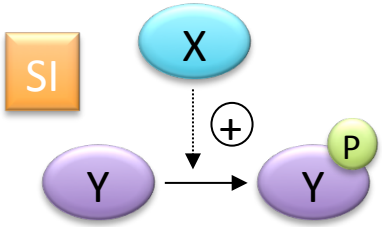

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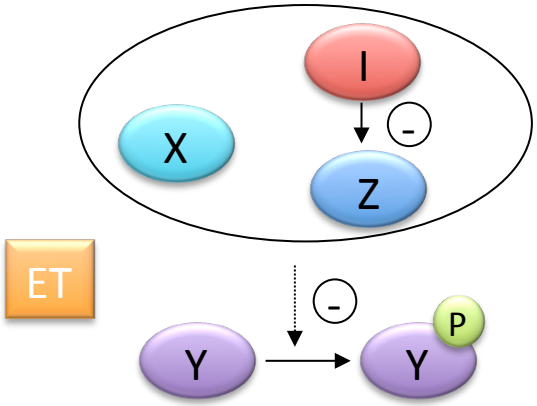

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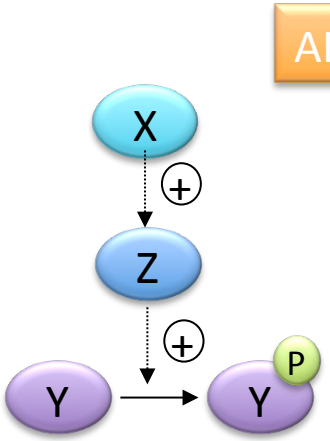
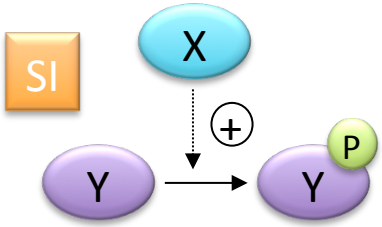


x active z et z phosphoryle y

Règles secondaires

Combiner les conclusions



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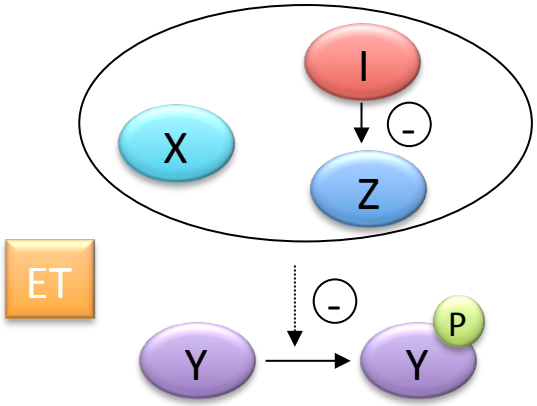

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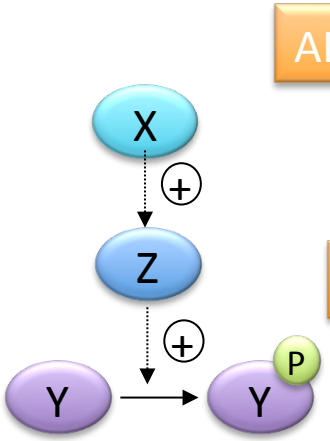

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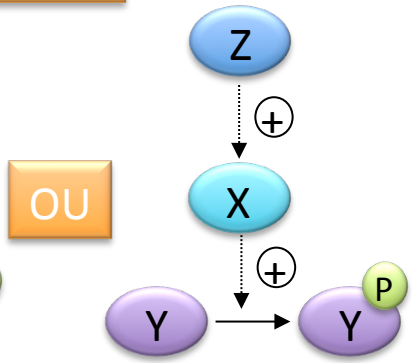

z active x et x phosphoryle y

Règles secondaires

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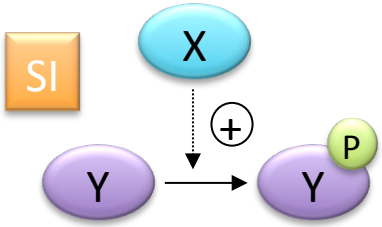

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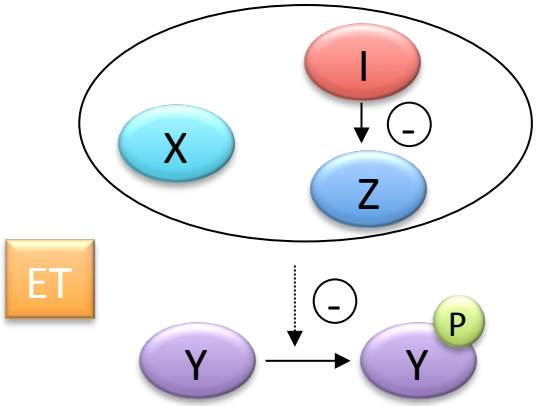

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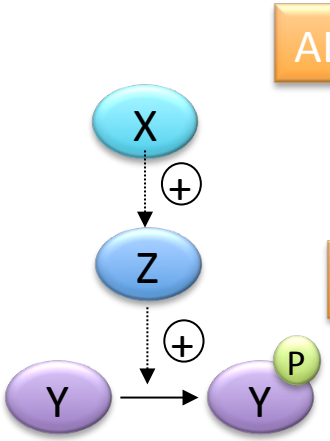
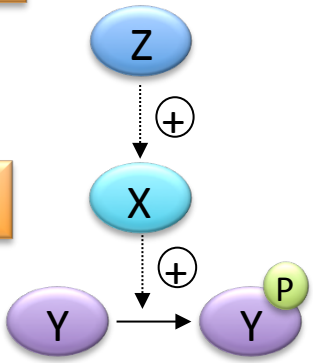


x active z et z phosphoryle y

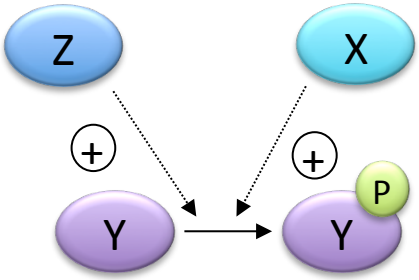
OU



z active x et x phosphoryle y

OU

x et z phosphorylent y de manière indépendante



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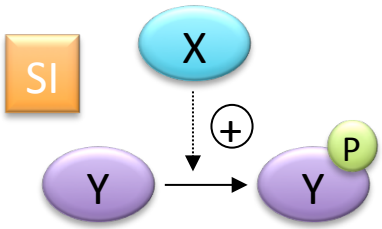

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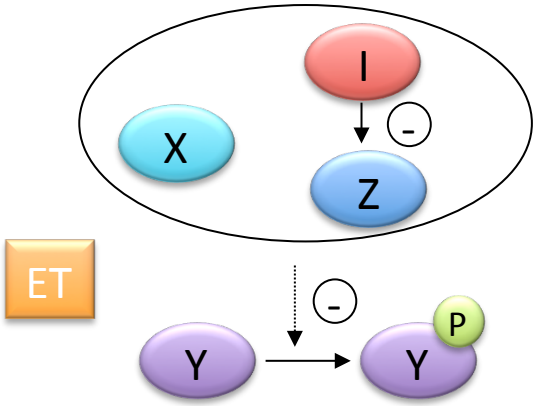

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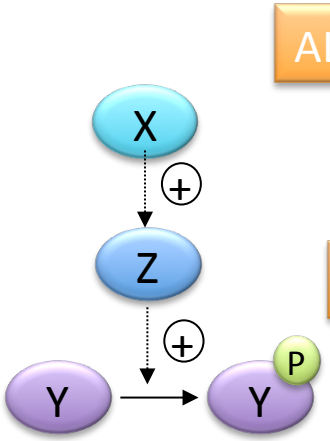

x active z et z phosphoryle y

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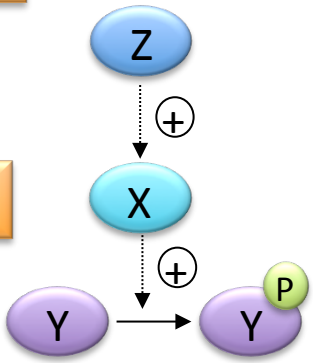
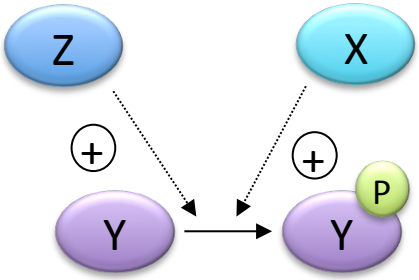


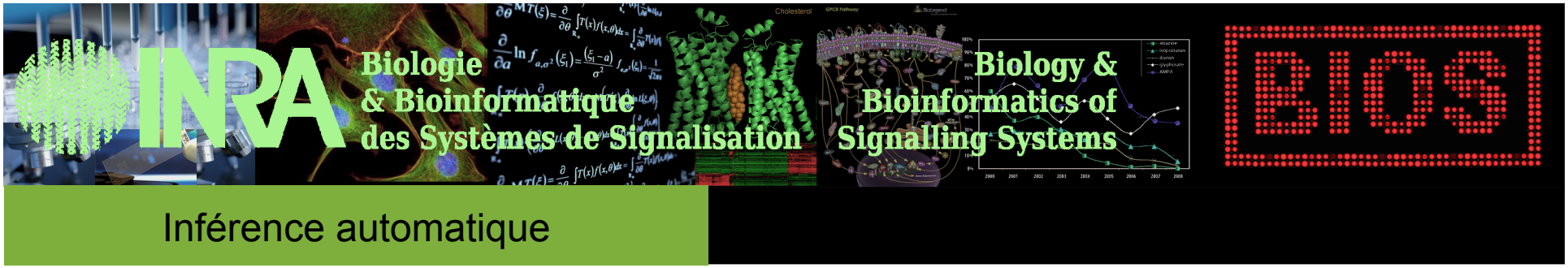
z active x et x phosphoryle y

OU

x et z phosphorylent y de manière indépendante



IF x phosphorylate y
AND inhibitor of z decreases phosphorylation
THEN (x activates z AND z phosphorylates y)
OR (z activates x AND x phosphorylates y)
OR (z phosphorylates y AND x phosphorylates y)



Inférence automatique

Test sur le réseau FSH.

Faits initiaux extraits de la bibliographie :

- ~ 150 articles
- ~ 250 expériences

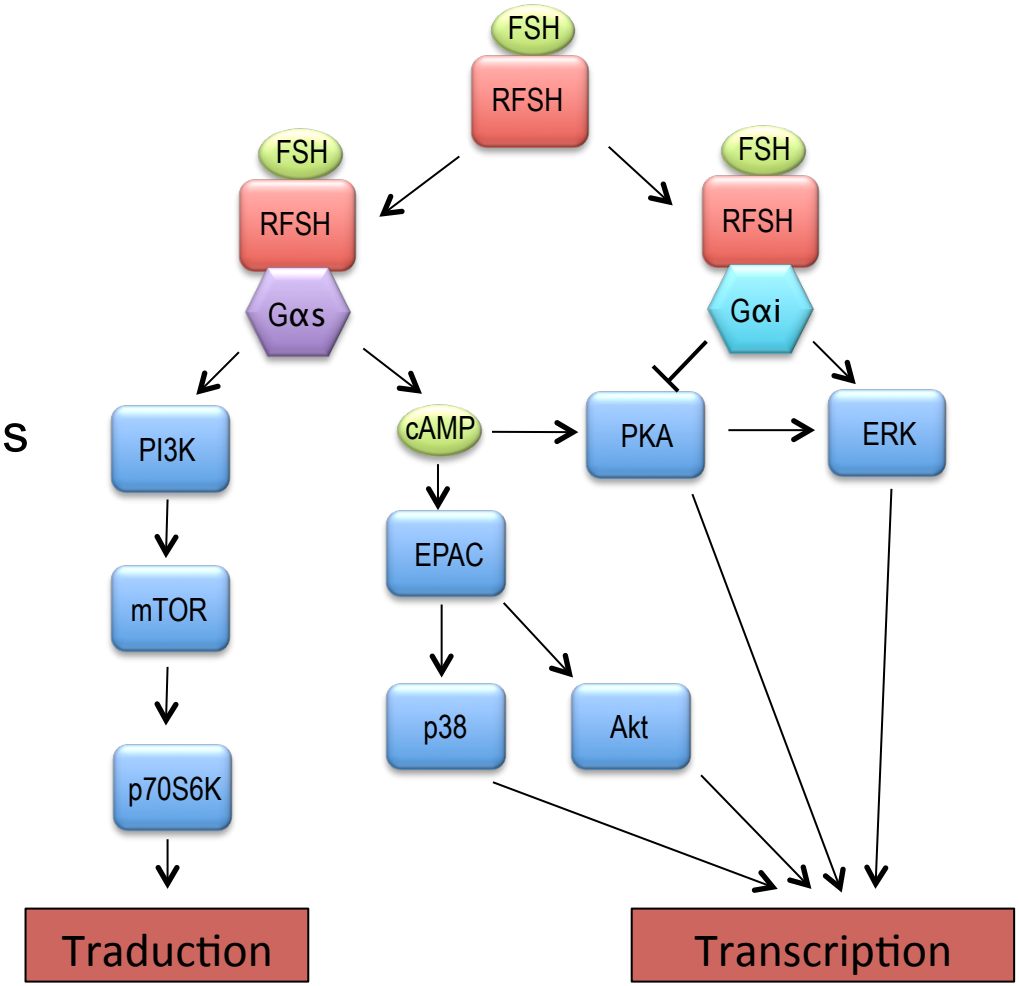
Actuellement l'extraction des expériences à partir des publications est manuelle !

Les données haut-débit peuvent être prises en compte automatiquement.

Inférence automatique

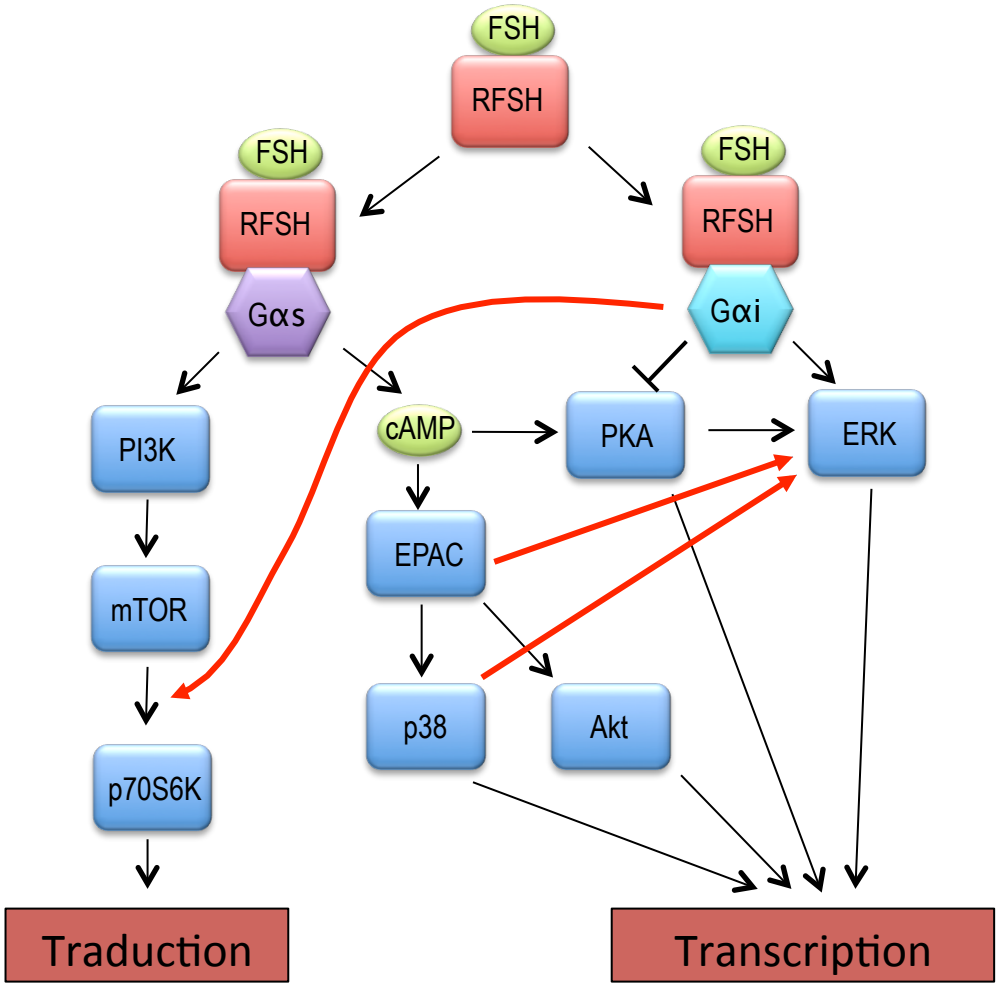
On retrouve l'essentiel du réseau attendu.

Cependant certaines relations attendues ne sont pas présentes, et effectivement ces relations ont été démontrées avec d'autres récepteurs.



Inférence automatique

Il y a également de nouvelles relations intéressantes trouvées



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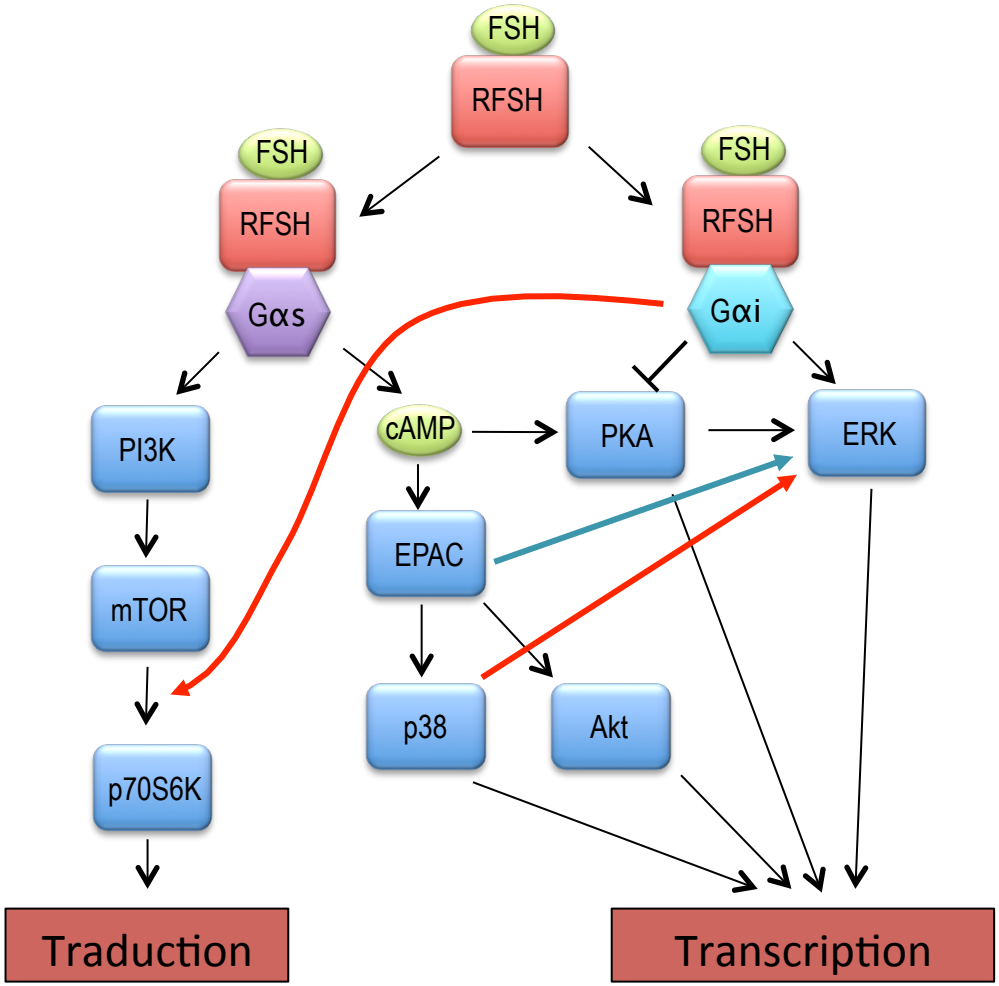

Inférence automatique

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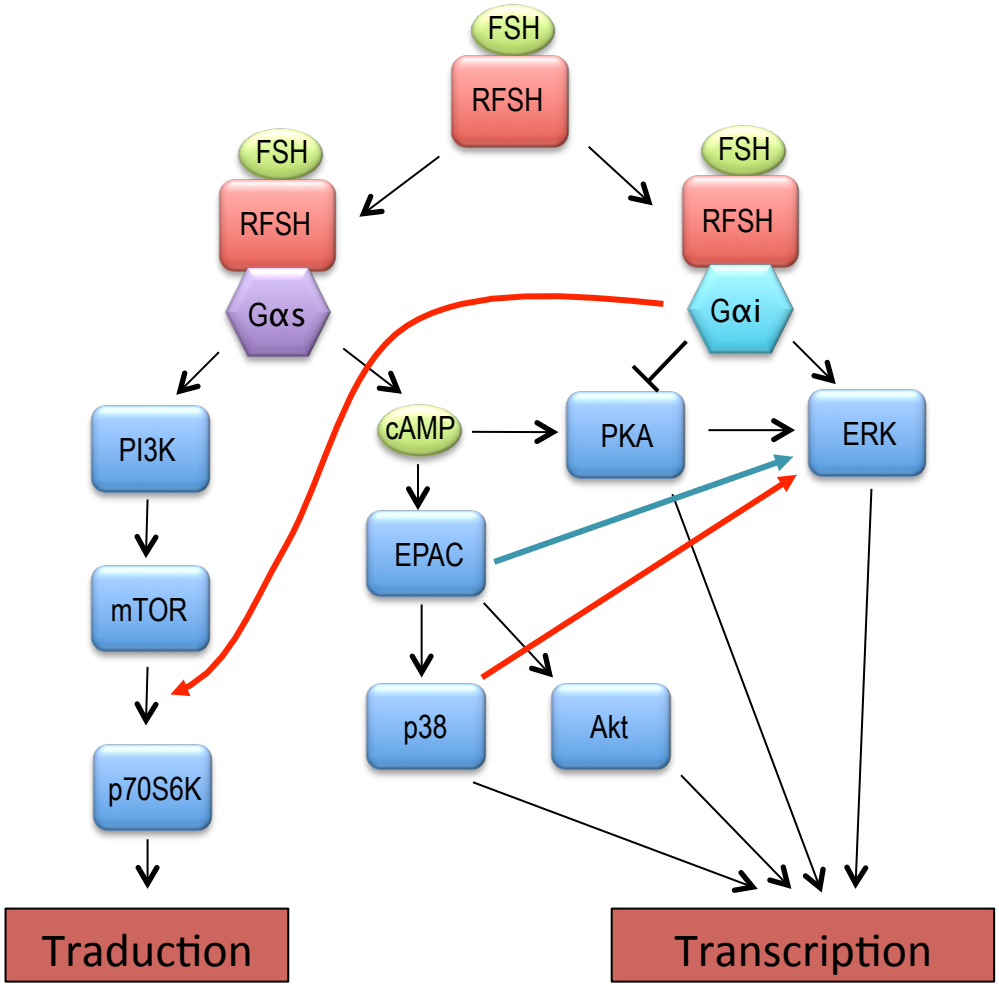
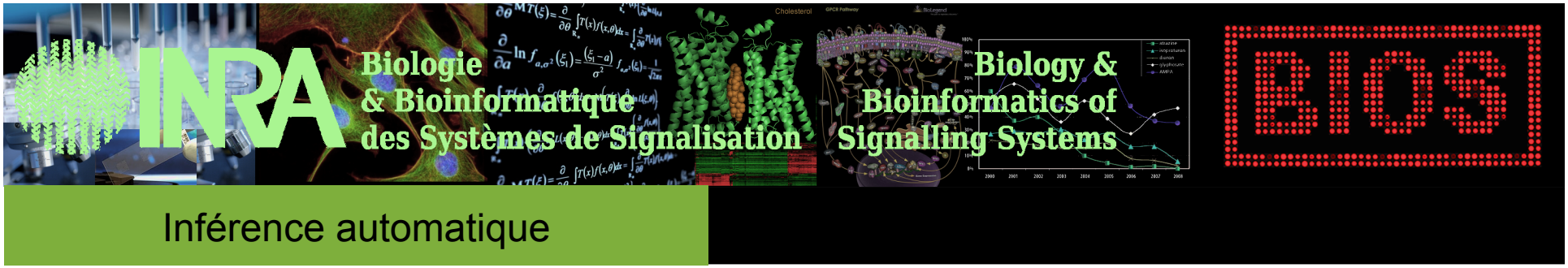
D'autres sont vraiment nouvelles. Validations expérimentales en cours.





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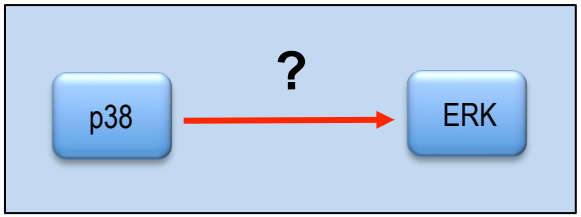

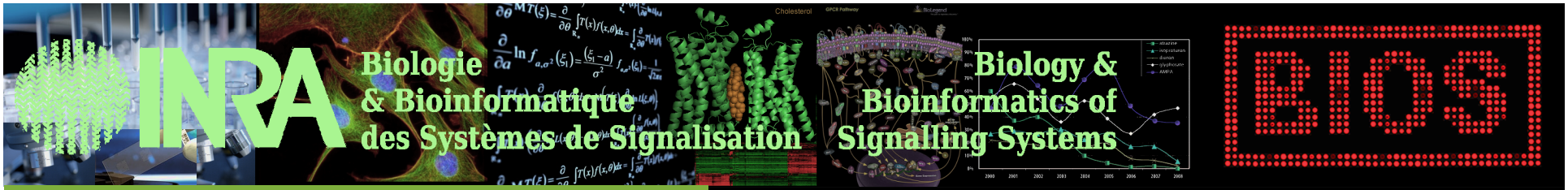

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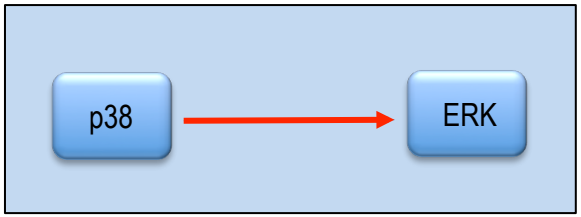
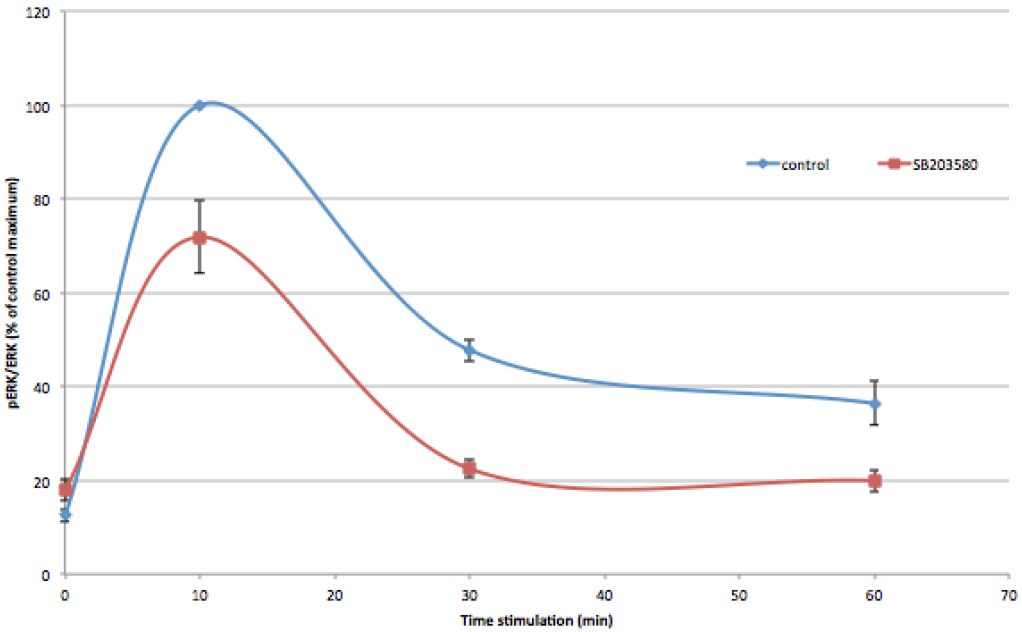
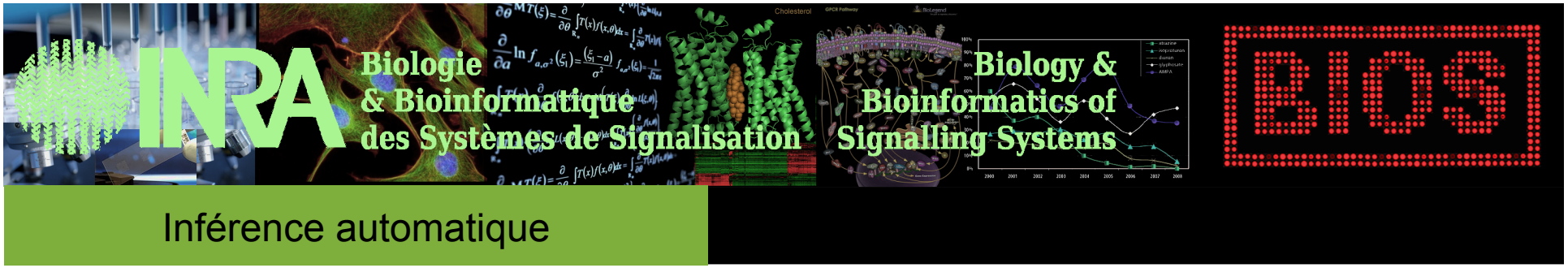


Validé !





Inférence automatique

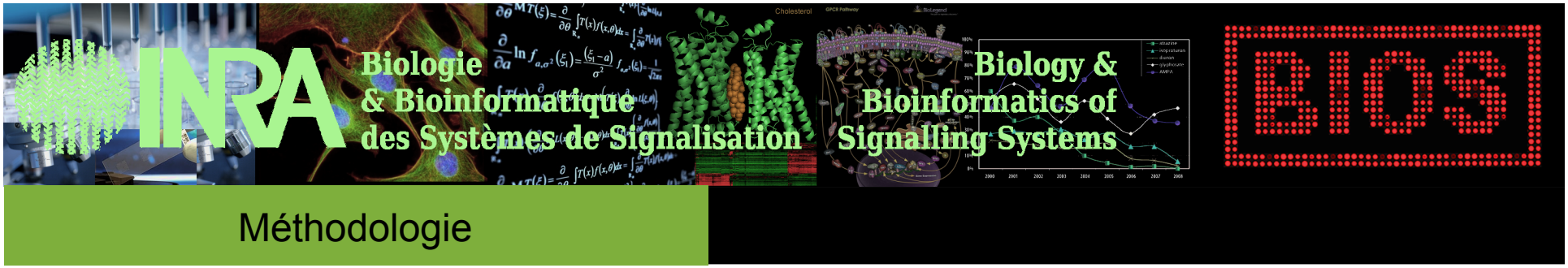
A partir d'un corpus d'expériences, nous sommes capables de reconstruire le réseau !

Mais :

L'extraction des expériences à partir de la littérature reste manuelle !

⇒ Automatiser l'extraction des expériences dans les articles

C'est l'objectif du projet Biosystémique !



Méthodologie

Un article « typique » de Biochimie/Biologie moléculaire

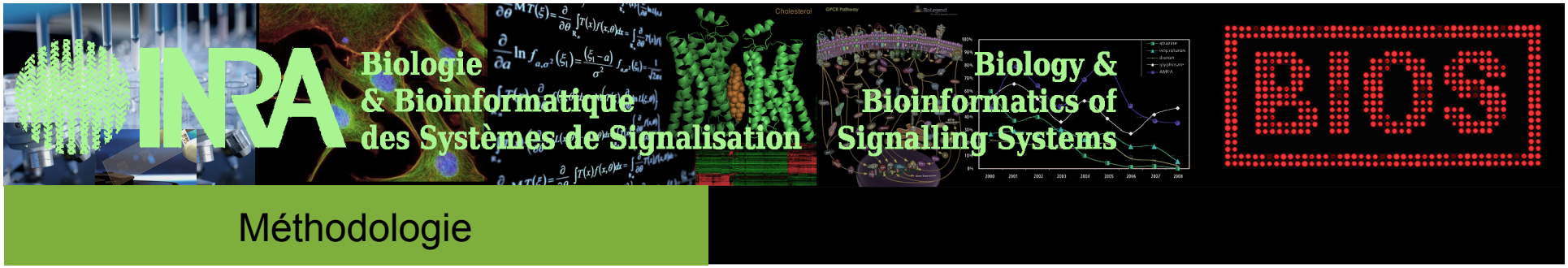
- Abstract : les principaux résultats

- Introduction
  Phénomène étudié
  Etat de l'art

- Résultats
  Liste et détail des expériences réalisées

- Discussion
  Interprétation des expériences en regard du phénomène étudié

- Conclusion



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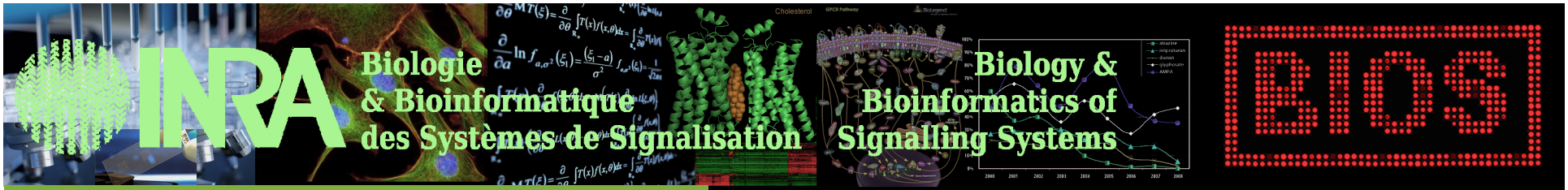
  Interprétation des expériences en regard du phénomène étudié

- Conclusion



Méthodologie

Annotation manuelle de quelques publications

Phosphoproteomics reveals new ERK MAP kinase targets and links ERK to nucleoporin-mediated nuclear transport

Hidetaka Kosako[1,2], Nozomi Yamaguchi[1,3], Chizuru Aranami[4], Masato Ushiyama[1,5], Shingo Kose[6], Naoko Imamoto[6], Hisaaki Taniguchi[2], Eisuke Nishida[7] & Seisuke Hattori[1,4]

Many extracellular signal–regulated kinase (ERK) mitogen-activated protein (MAP) kinase substrates have been identified, but the diversity of ERK-mediated processes suggests the existence of additional targets. Using a phosphoproteomic approach combining the steroid receptor fusion system, IMAC, 2D-DIGE and phosphomotif-specific antibodies, we detected 38 proteins showing reproducible phosphorylation changes between ERK-activated and ERK-inhibited samples, including 24 new candidate ERK targets. ERK directly phosphorylated at least 13 proteins *in vitro*. Of these, Nup50 was verified as a bona fide ERK substrate. Notably, ERK phosphorylation of the FG repeat region of Nup50 reduced its affinity for importin-β family proteins, importin-β and transportin. Other FG nucleoporins showed a similar functional change after ERK-mediated phosphorylation. Nuclear migration of importin-β and transportin was impaired in ERK-activated, digitonin-permeabilized cells, as a result of ERK phosphorylation of Nup50. Thus, we propose that ERK phosphorylates various nucleoporins to regulate nucleocytoplasmic transport.

Phosphorylation by protein kinases is the most widespread type of post-translational modification used in signal transduction and can regulate many properties of a protein. The global identification and characterization of the *in vivo* substrates of an individual protein kinase is essential for a thorough and therapeutically applicable understanding of its cellular functions in physiological and pathological states. Extensive studies have revealed that kinase-substrate interaction, subcellular localization and catalytic specificity are all involved in substrate phosphorylation by a kinase in cells[1]. Identification of candidate substrates for a specific kinase has proved difficult because of the transient nature of kinase-substrate interaction and the limited substrate sequence specificity of kinases.

To date, a number of methodologies have been developed to identify bona fide substrates of protein kinases[2]. *In vitro* phosphorylation screens digested phosphopeptide, and it is difficult to determine the stoichiometry of phosphorylation. Two-dimensional gel electrophoresis (2D-E) is a classical but still powerful tool for proteomics[10–12]. Because protein phosphorylation causes a shift in its isoelectric point, 2D-E is suitable for detecting quantitative changes in phosphorylation, which appear as distinct spot trains along the horizontal axis.

Prefractionation methods have been shown to be effective for proteomic approaches to the analysis of proteins of low abundance, such as signaling molecules. One of these methods, immobilized metal ion–affinity chromatography (IMAC), can enrich not only phosphopeptides but also phosphoproteins from cell lysates[13–15]. We recently developed a preliminary phosphoproteomic approach that combines phosphoprotein enrichment by IMAC and fluorescent two-dimensional difference gel electrophoresis (2D-DIGE) technology[16].

Dans la partie « Résultats », on identifie les phrases essentielles : celles qui donnent les résultats des expériences

this protein could also be a direct substrate under some conditions.

ERK phosphorylates Nup50 at Ser221 and Ser315

We then focused on Nup50 (also called Npap60), a component of the NPC, for the following reasons. First, ERK activation induced a marked acidic shift in the isoelectric point of endogenous Nup50, suggesting highly stoichiometric phosphorylation (**Fig. 2b**). Second, Nup50 is efficiently phosphorylated by ERK *in vitro* (**Figs. 3a** and **4a**). Third, activated ERK is reported to inhibit nuclear protein import[31]. Fourth, ERK enters the nucleus via direct interaction with NPC proteins[32,33]. Nup50 seems to function at the terminal stages of nuclear protein import to coordinate import complex disassembly and importin recycling[34]. It has also been reported that Nup50 acts as a cofactor for importin-α–importin-β–mediated nuclear import to stimulate classical nuclear localization signal (NLS)-directed import[35]. Nup50 contains an N-terminal domain that binds to importin-α (N), a central FG repeat domain that binds to importin-β (FG) and a C-terminal Ran-binding domain (R)[35]. ERK phosphorylated Nup50 exclusively in the FG repeat domain *in vitro* (**Fig. 4a**). LC-MS and tandem MS (MS/MS) analyses of ERK-phosphorylated and unphosphorylated GST-tagged full-length Nup50 demonstrated that ERK phosphorylates Nup50 stoichiometrically at Ser221 and Ser315 *in vitro* (**Supplementary Fig. 3**). *In vitro* kinase assays with wild-type and Ser-to-Ala mutants of Nup50 confirmed this finding (**Fig. 4b**).

To examine whether Ser221 and Ser315 of Nup50 are directly phosphorylated by ERK *in vivo*, we generated phospho-specific (**Fig. 4c**). We stimulated serum-starved HeLa cells with epidermal growth factor (EGF) or TPA (12-O-tetradecanoylphorbol-13-acetate) in the presence or absence of U0126 and then probed the cell lysates with these phospho-specific antibodies. EGF and TPA induced the phosphorylation of endogenous Nup50 at Ser221 and Ser315, along with ERK1/2 activation, and all were blocked by U0126 pretreatment (**Fig. 4d**). We then investigated the subcellular localization of phosphorylated Nup50 in TPA-stimulated HeLa cells by indirect immunofluorescence microscopy. Ser221-phosphorylated Nup50 appeared in the nucleus after TPA treatment (anti–pS315-Nup50 did not show specific staining) (**Fig. 4e**, middle). This TPA-induced nuclear staining was abolished by small interfering RNA (siRNA)-mediated depletion of Nup50 (**Supplementary Fig. 4**), confirming that this staining was not nonspecific, but indeed reflected the phosphorylation of Nup50 at Ser221. U0126 pretreatment abolished the staining of TPA-treated interphase cells with the anti–pS221-Nup50 antibody (**Fig. 4e**, right). Notably, in untreated cells and cells treated with U0126 plus TPA, we observed strong Nup50 phosphorylation at Ser221 only in mitotic cells (indicated by the arrowheads in **Fig. 4e** and the arrow in **Supplementary Fig. 4b**). Ser221 fits the minimal Cdk1 consensus motif S/T-P; in fact, Cdk1–cyclin B phosphorylated Nup50 at Ser221 *in vitro* (**Supplementary Fig. 5**). Therefore, all these results suggest that both ERK and Cdk1 directly phosphorylate Nup50 at Ser221 in intact cells.

Because phosphorylation of nucleoporins is suggested to cause disassembly of the NPC during mitosis[27], and because both ERK and

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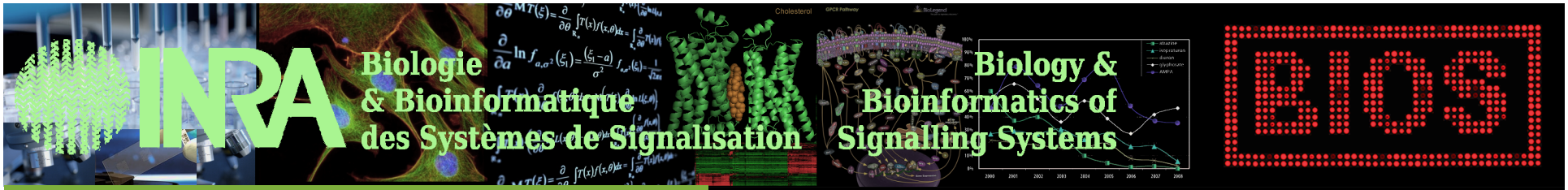

Méthodologie

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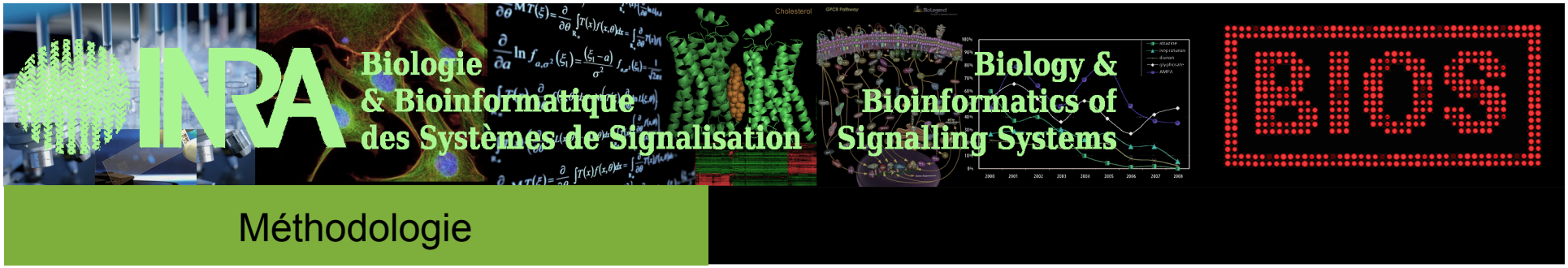

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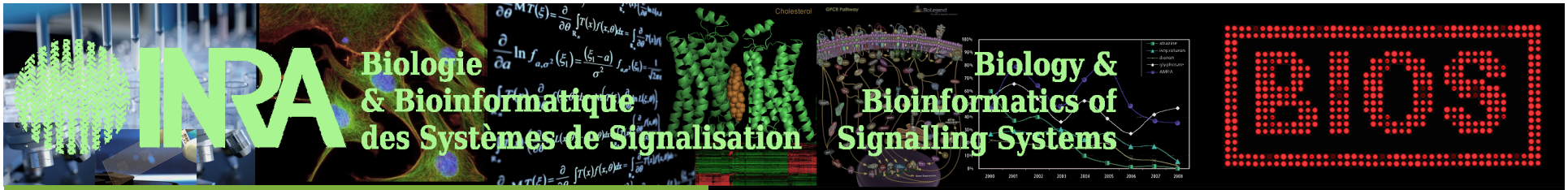

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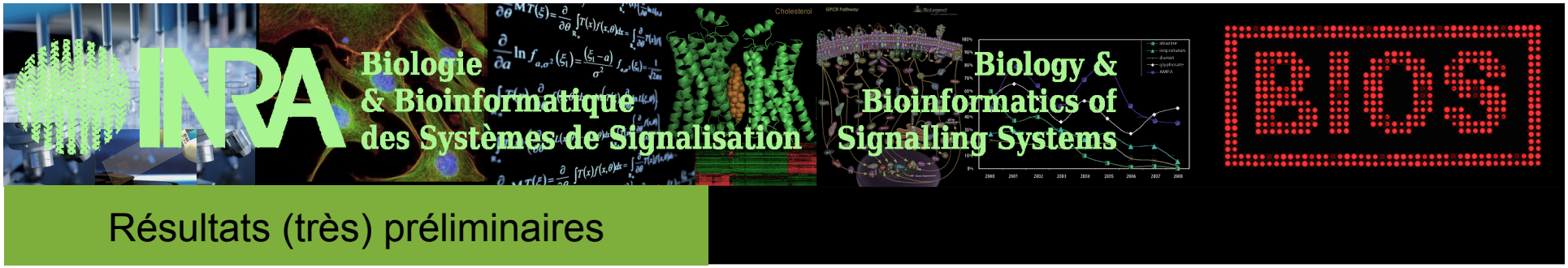
Résultat attendu : à partir d'un article, extraction de 10-15 phrases hautement informatives.

⇒ permet d'évaluer très vite le contenu de l'article.

Avantages par rapport aux méthodes de text mining qui travaillent sur les abstracts et/ou la discussion :

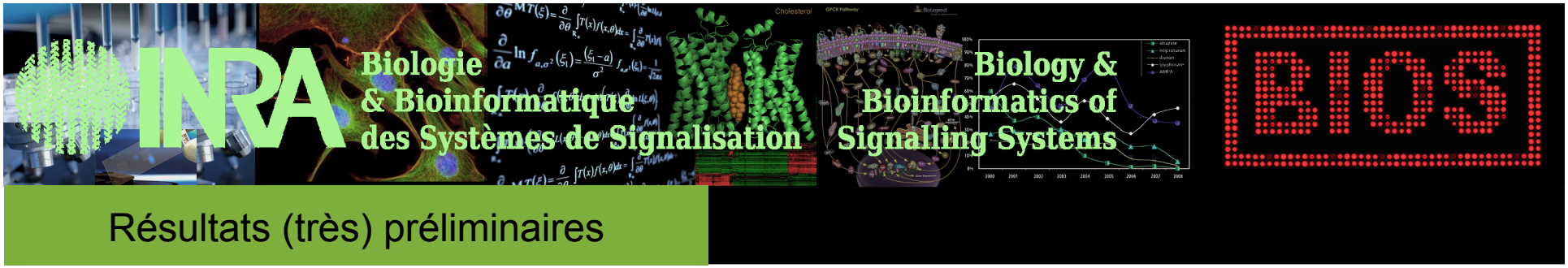
- Moins de biais (résultats expérimentaux non interprétés)
- Moins de bruit (on ne considère ni l'introduction ni les références biblio)
- Plus complet car toutes les expériences ne sont pas reprises dans l'abstract et/ou la discussion.



Résultats (très) préliminaires

On utilise les cascades CasSys.

- Première étape : créer des dictionnaires.
  - Molécules : extraction à partir de bases de données
    - Une même molécule a de nombreux noms, plus ou moins bien orthographiés !
    - Une protéine peut être désigné par le nom du gène correspondant.
  - Verbes
    - de démonstration : confirm, demonstrate, detect, find, provide...
    - d'action : activate, enhance, increase, induce...
  - Méthodes expérimentales : western blot, mass spectrometry, etc. Ici encore pas mal de variations possibles pour une même méthode, utilisation d'acronymes...



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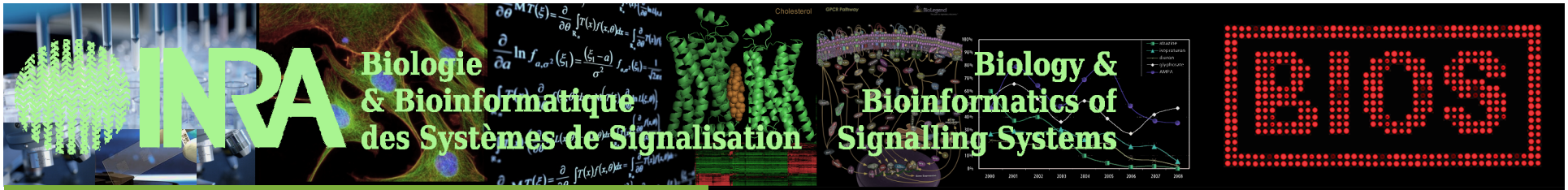
- Deuxième étape : créer des graphes



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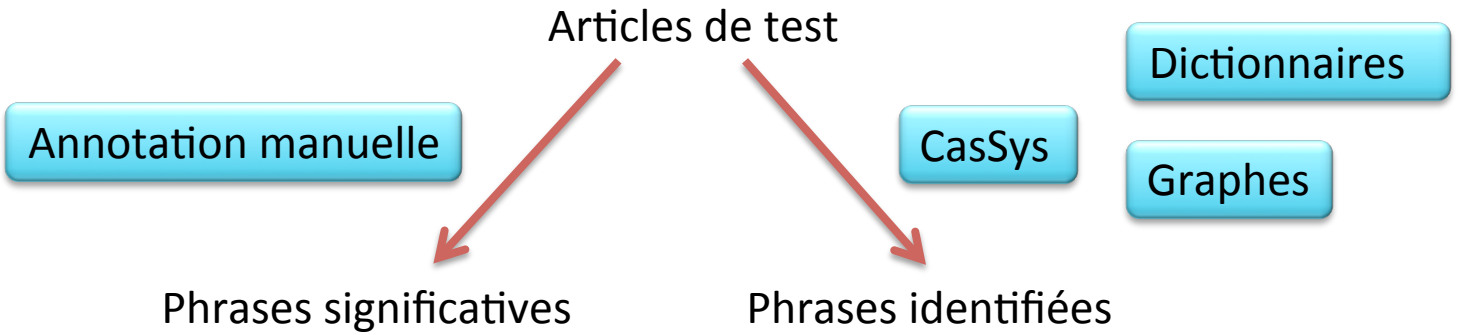

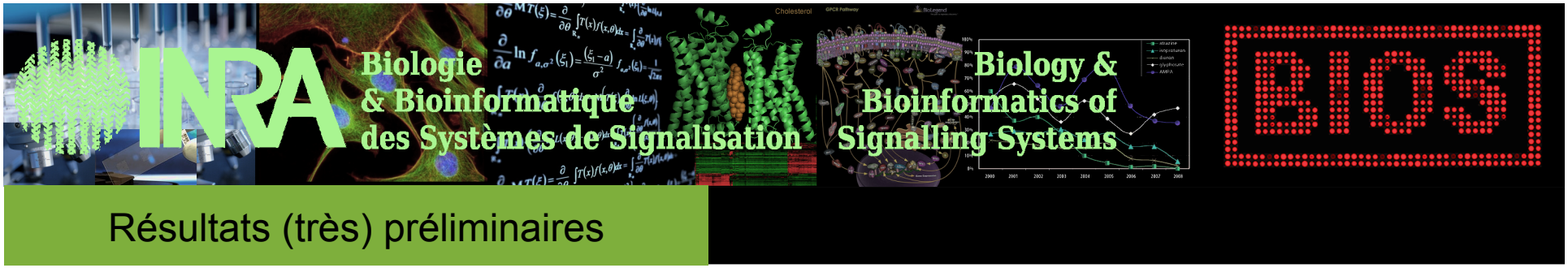

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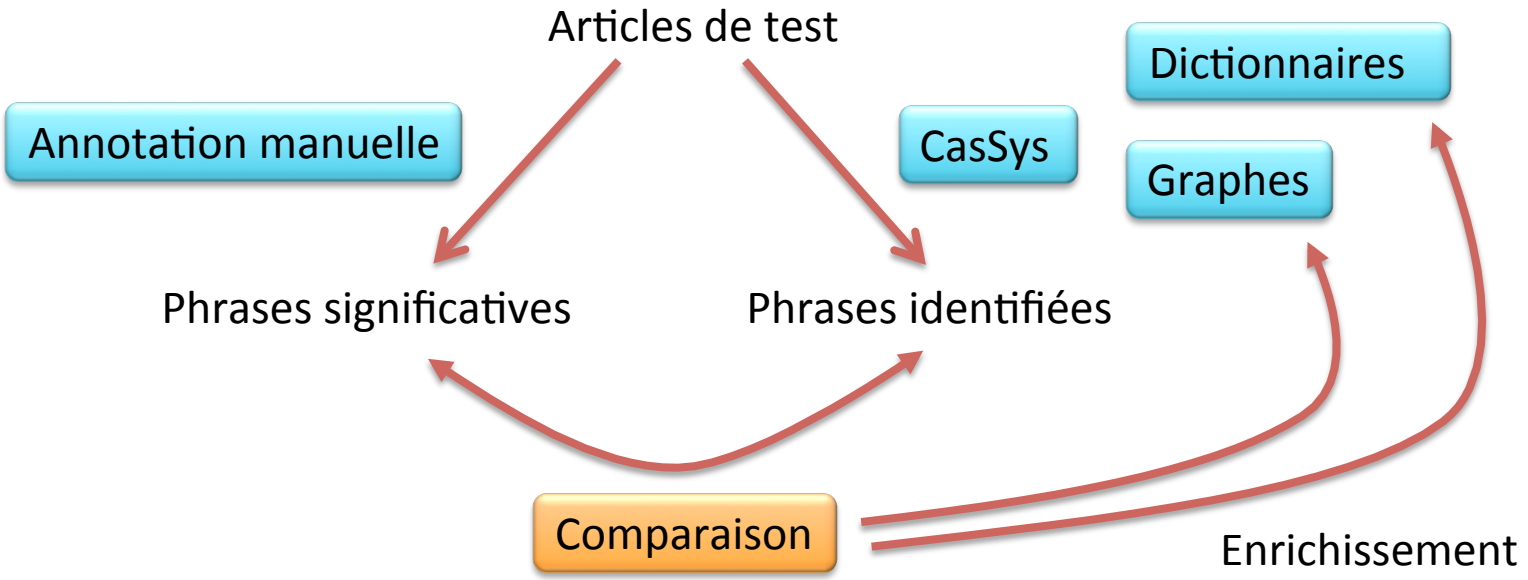
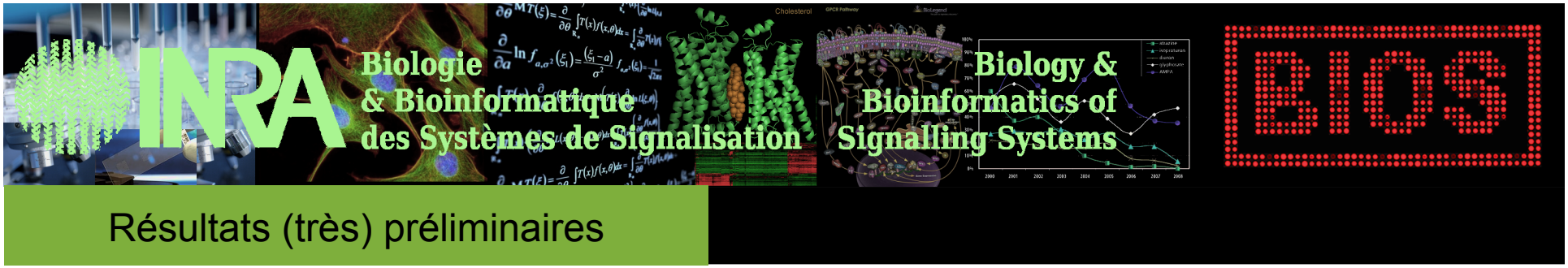




Résultats (très) préliminaires

Article de test 1

- 14 groupes de phrases significatives
- Avec notre premier graphe on retrouve 6 groupes

JOURNAL OF CELLULAR PHYSIOLOGY 204:437–444 (2005)

Cdc25A and ERK interaction: EGFR-Independent ERK Activation by a Protein Phosphatase Cdc25A Inhibitor, Compound 5

ZIQIU WANG, BAOCHUN ZHANG, MEIFANG WANG, AND BRIAN I. CARR*
Thomas E. Starzl Transplant Institute, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Extracellular signal-regulated kinase (ERK) plays a central role in regulating cell growth, differentiation, and apoptosis. We previously found that 2-(2-mercaptoethanol)-3-methyl-1,4-napthoquinone or Compound 5 (Cpd 5), is a Cdc25A protein phosphatase inhibitor and causes prolonged, strong ERK phosphorylation which is triggered by epidermal growth factor receptor (EGFR) activation. We now report that Cpd 5 can directly cause ERK phosphorylation by inhibiting Cdc25A activity independently of the EGFR pathway. We found that Cdc25A physically interacted with and de-phosphorylated phospho-ERK both in vitro and in cell culture. Inhibition of Cdc25A activity by Cpd 5 resulted in ERK hyper-phosphorylation. Transfection of Hep3B human hepatoma cells with inactive Cdc25A mutant enhanced Cpd 5 action on ERK phosphorylation, whereas over-expression of Cdc25A attenuated this Cpd 5 action. Furthermore, endogenous Cdc25A knock-down by Cdc25A siRNA resulted in a constitutive-like ERK phosphorylation and Cpd 5 treatment further enhanced it. In EGFR-devoid NR6 fibroblasts and MEK (ERK kinase) mutated MCF7 cells, Cpd 5 treatment also resulted in ERK phosphorylation, providing support for the idea that Cpd 5 can directly act on ERK phosphorylation by inhibiting Cdc25A activity. These data suggest that phospho-ERK is likely another Cdc25A substrate, and Cpd 5-caused ERK phosphorylation is probably regulated by both EGFR-dependent and EGFR-independent pathways. J. Cell. Physiol. 204: 437–444, 2005.

Article de test 2

- 15 groupes de phrases significatives
- On en retrouve 6

Differentiation (2007) 75:299–307 DOI: 10.1111/j.1432-0436.2006.00143.x Journal compilation © 2007, International Society of Differentiation

ORIGINAL ARTICLE

Jian Li · Guangwen Wang · Chengyan Wang · Yang Zhao · Hong Zhang · Zhijia Tan · Zhihua Song · Mingxiao Ding · Hongkui Deng

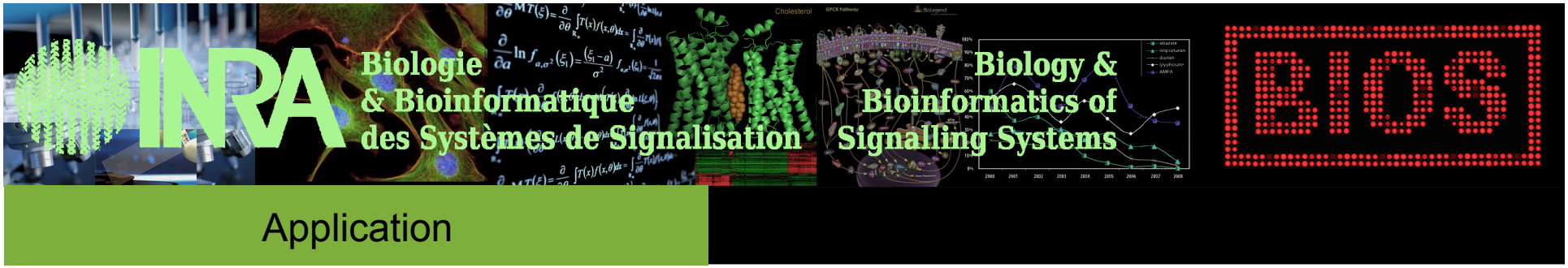
MEK/ERK signaling contributes to the maintenance of human embryonic stem cell self-renewal

Received April 6, 2006; accepted in revised form August 14, 2006

Abstract MEK/ERK signaling plays a crucial role in a diverse set of cellular functions including cell proliferation, differentiation and survival, and recently has been reported to negatively regulate mouse embryonic stem cell (mESC) self-renewal by antagonizing STAT3 activity. However, its role in human ESCs (hESCs) remains unclear. Here we investigated the functions of MEK/ERK in controlling hESC activity. We demonstrated that MEK/ERK kinases were targets of fibro- make evident the striking differences in the control of self-renewal between hESCs and mESCs.

Key words human embryonic stem cells · self-renewal · MEK/ERK · FGF

Introduction



Application

Dans le cadre du projet : application à la recherche de substrats de ERK ß-arrestine-dépendants, démonstration de la spécificité vis-à-vis du récepteur.

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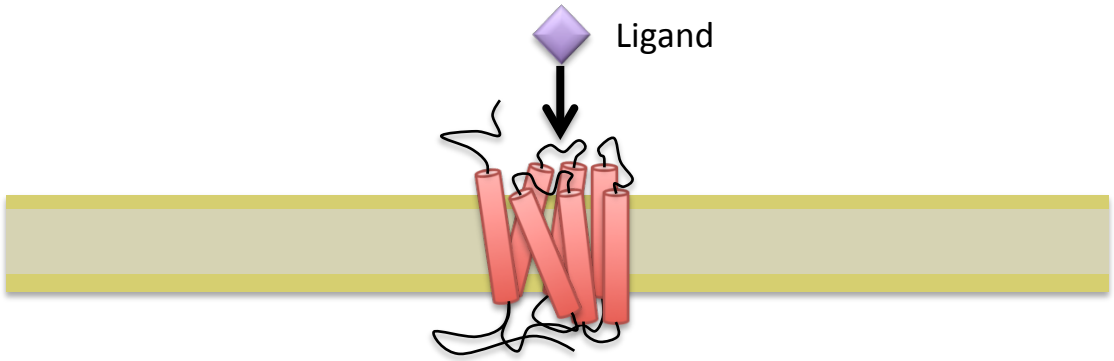

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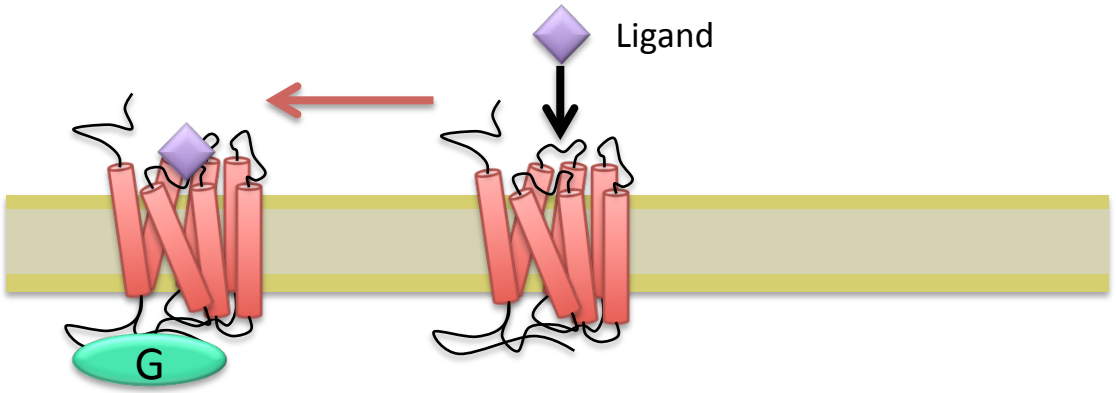

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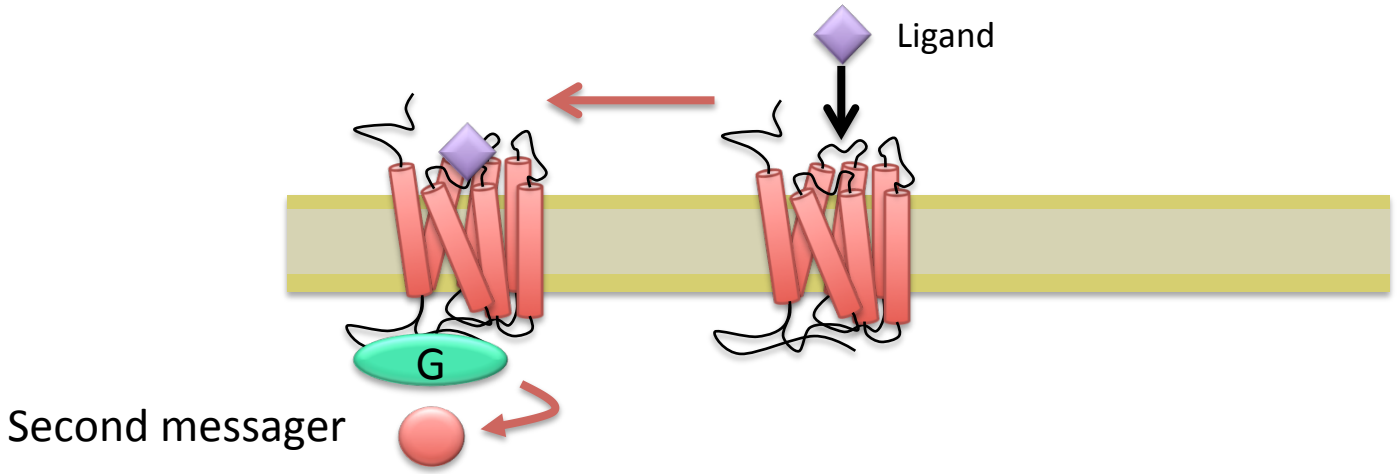

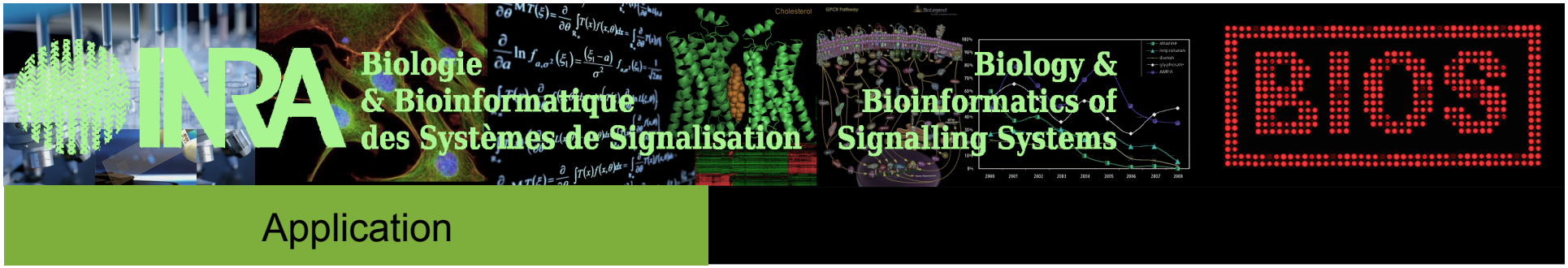

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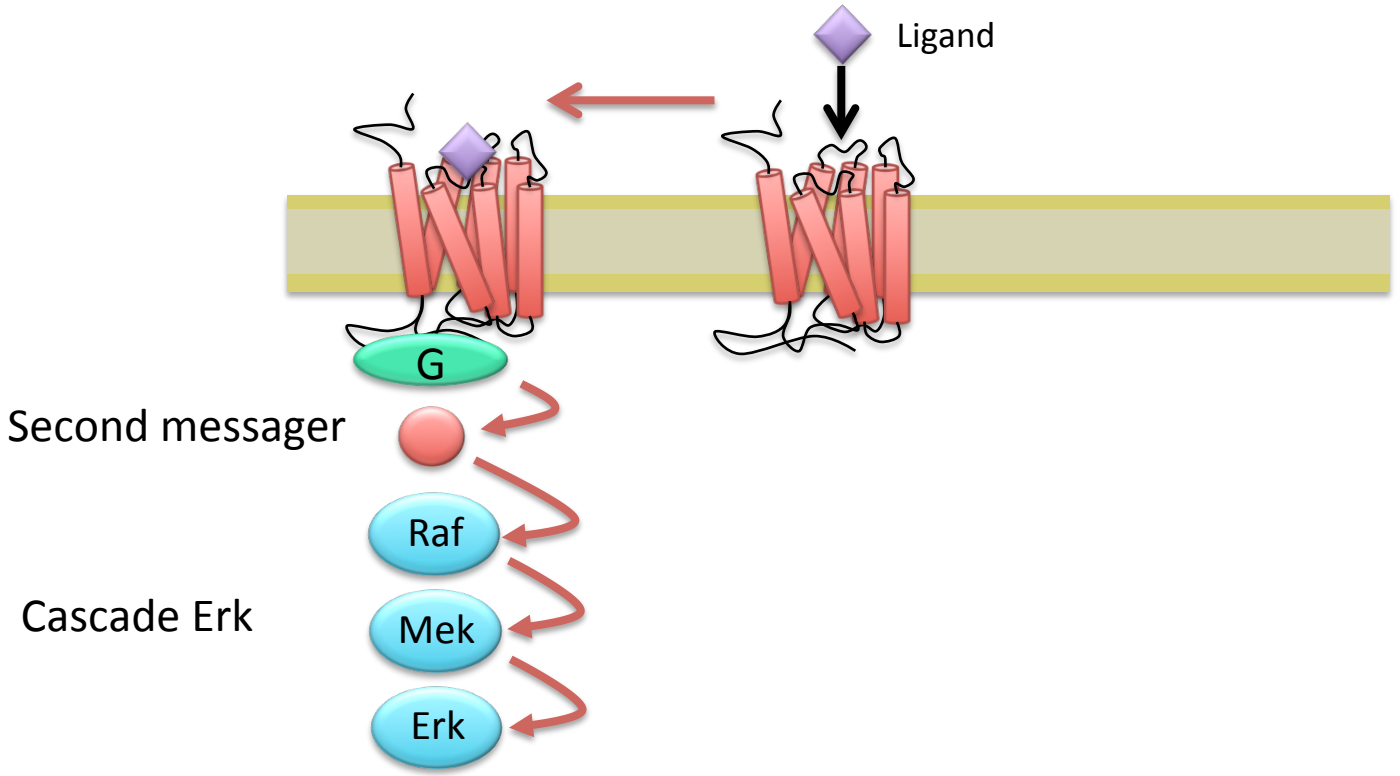

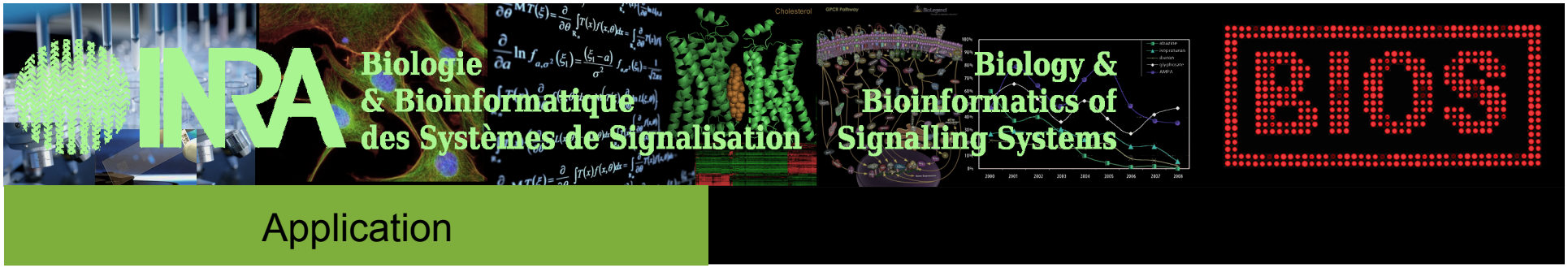

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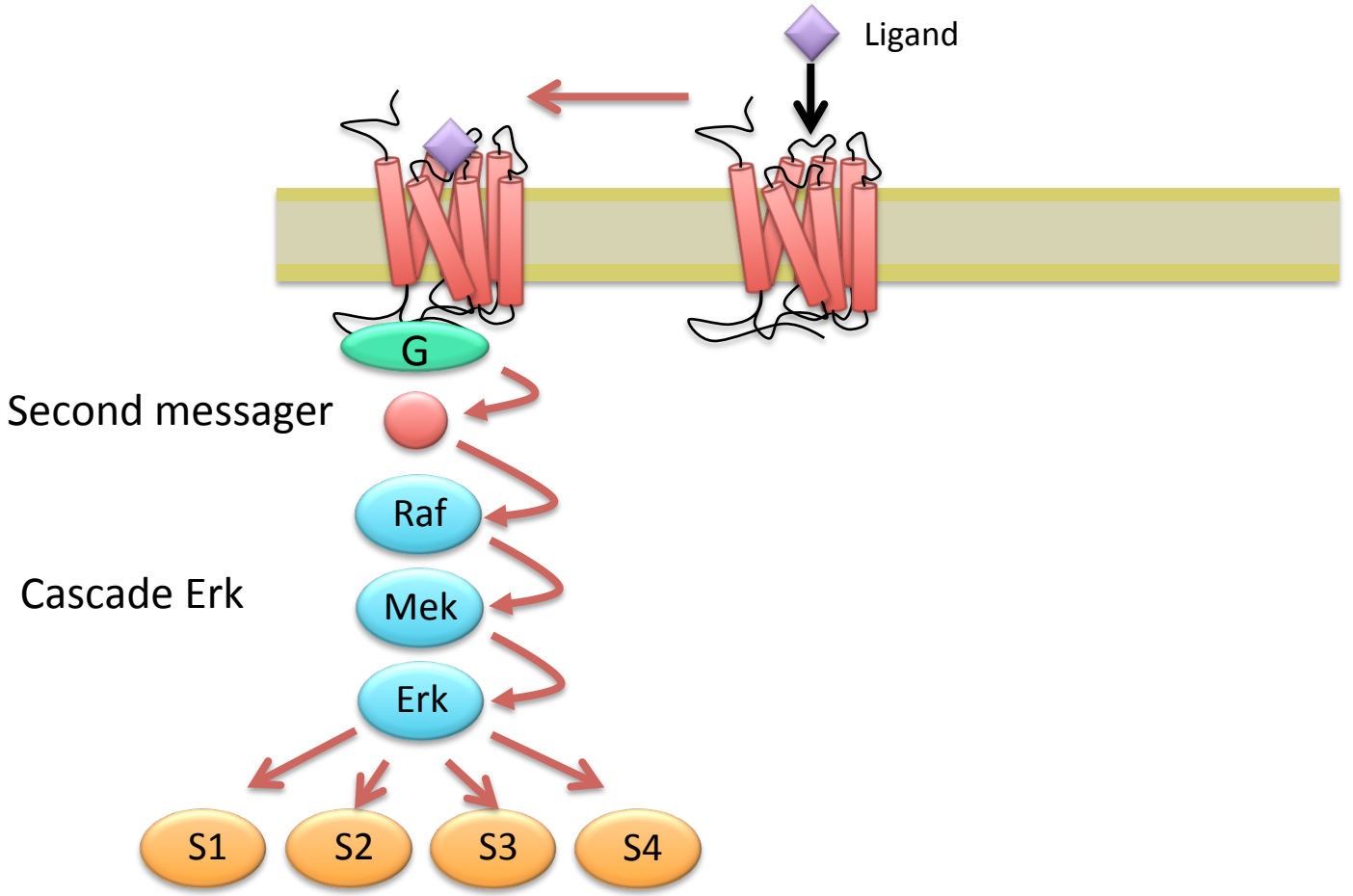

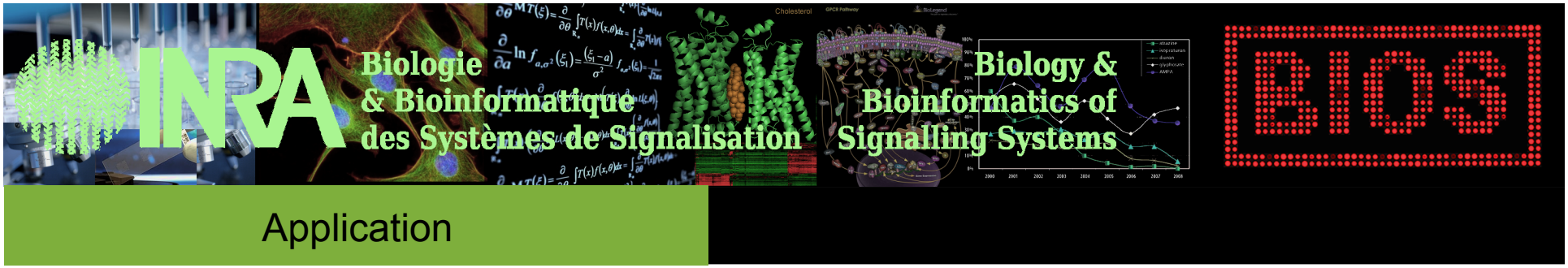

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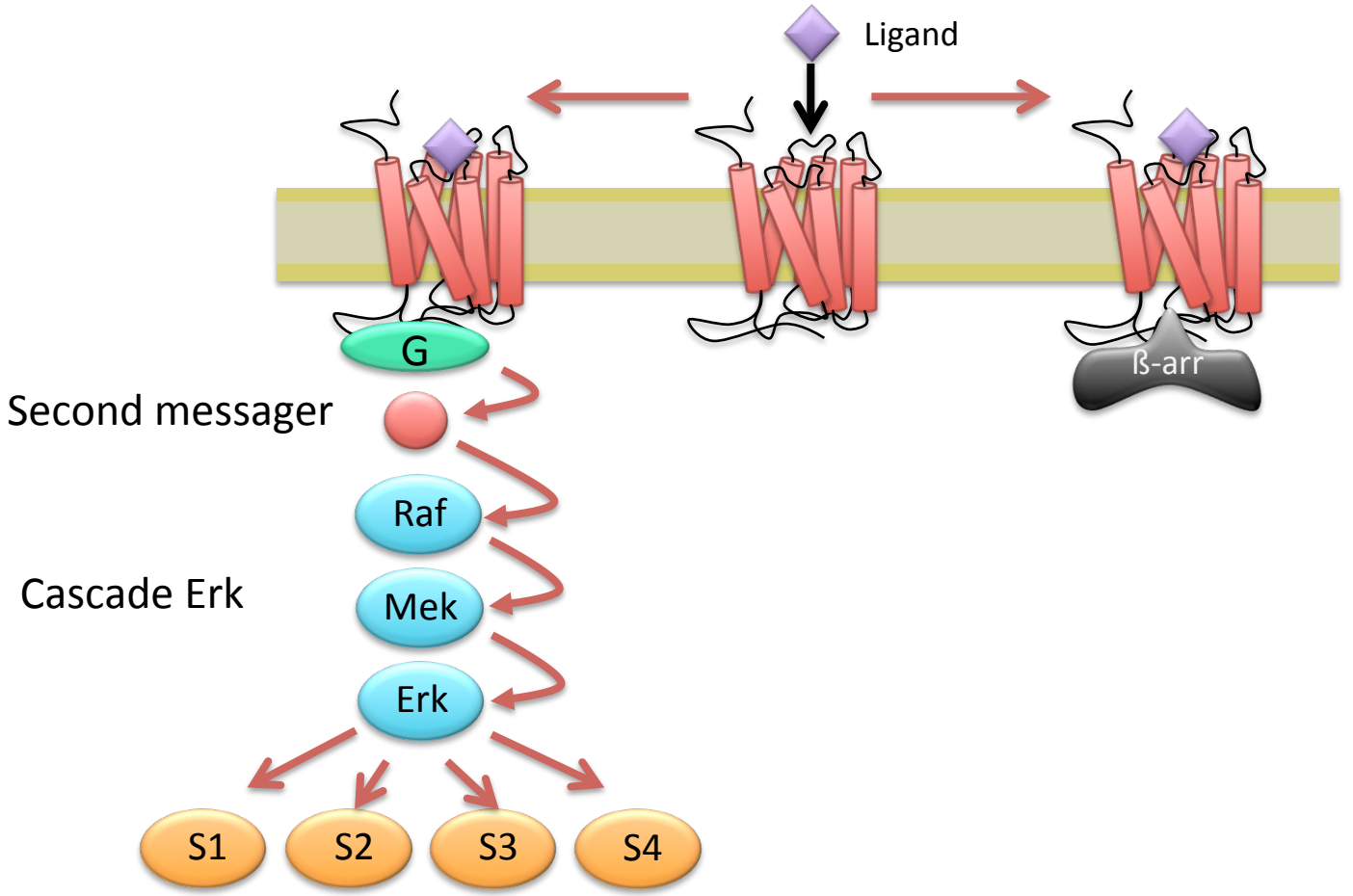

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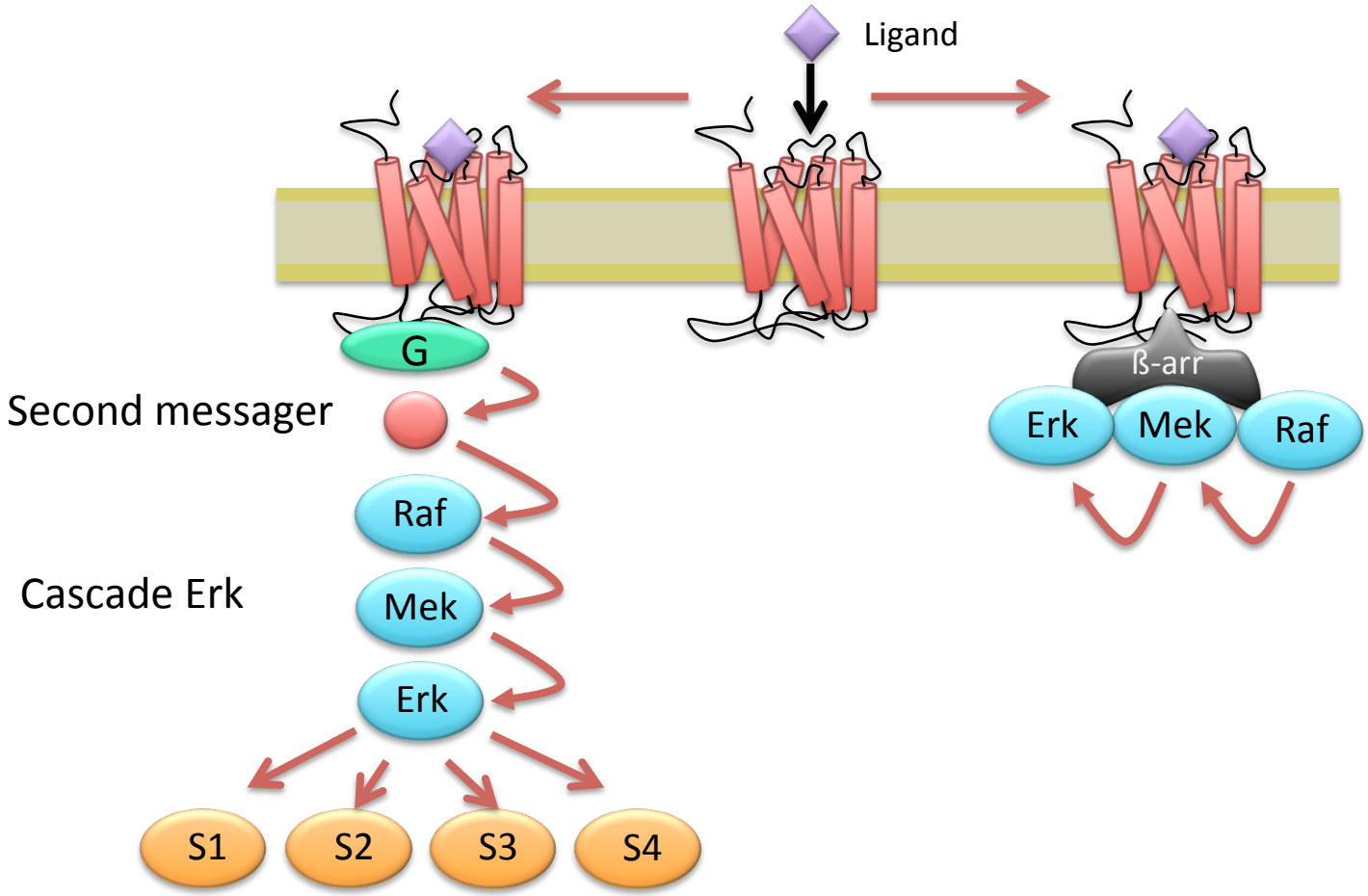

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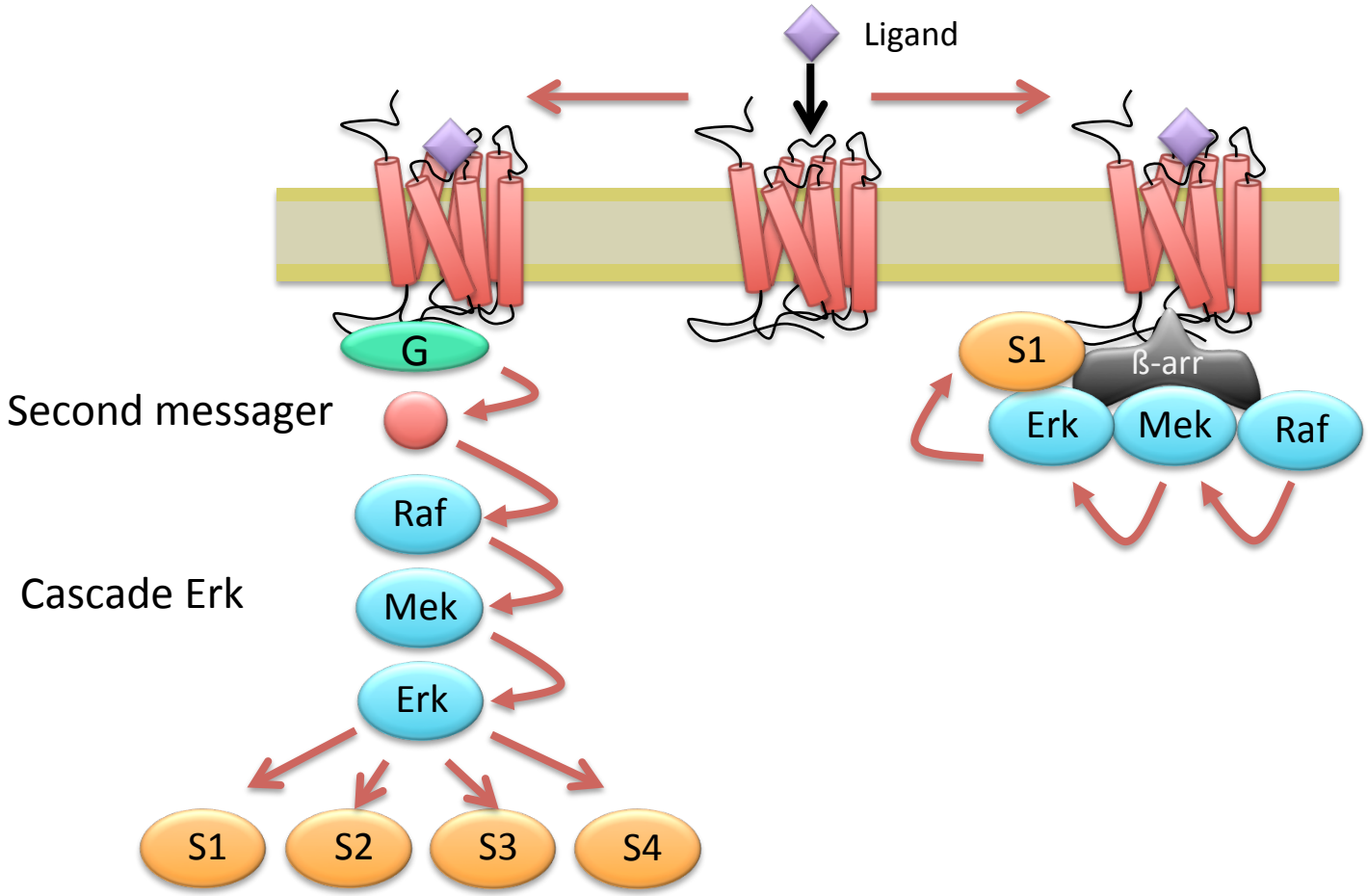

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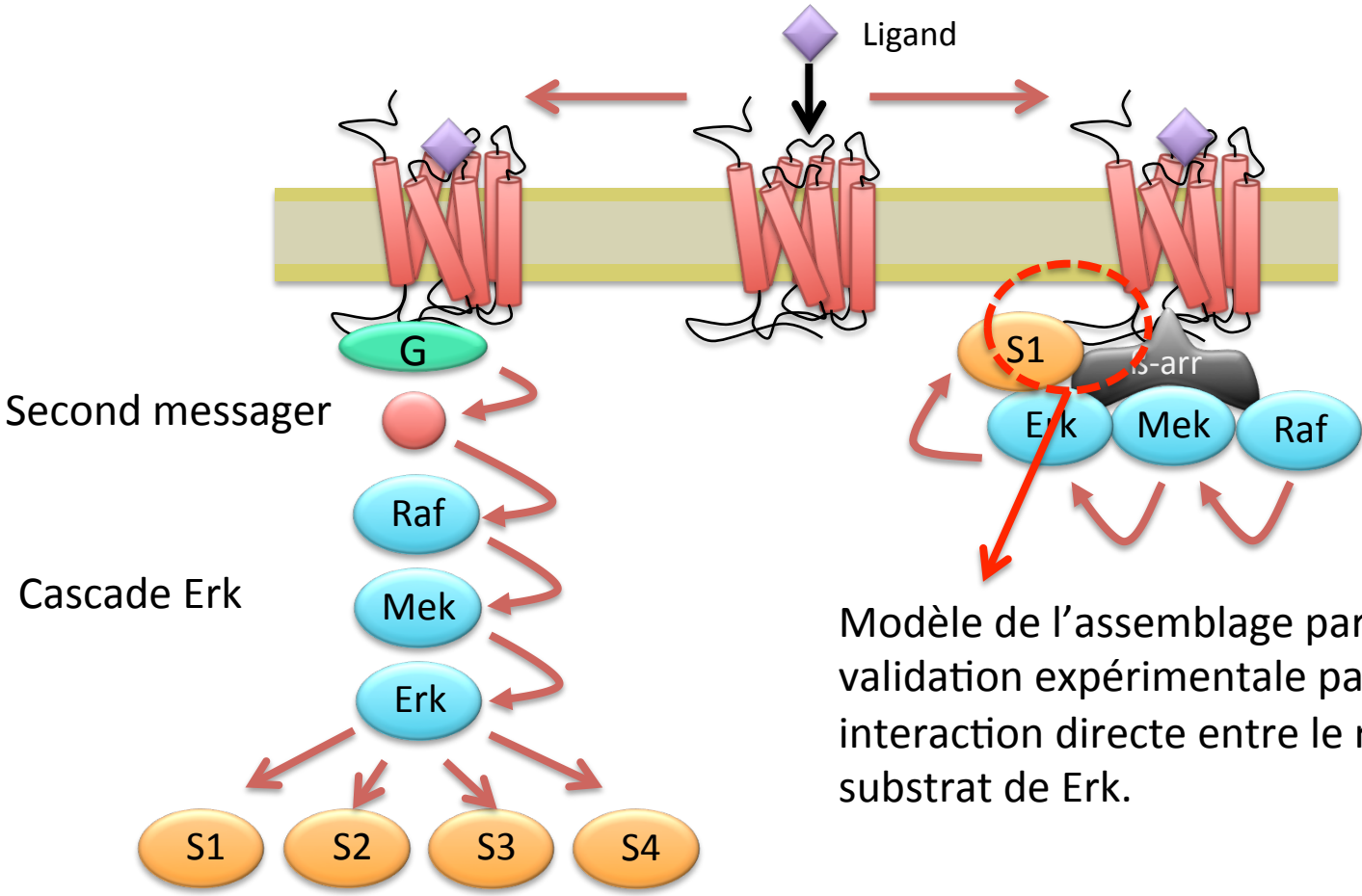
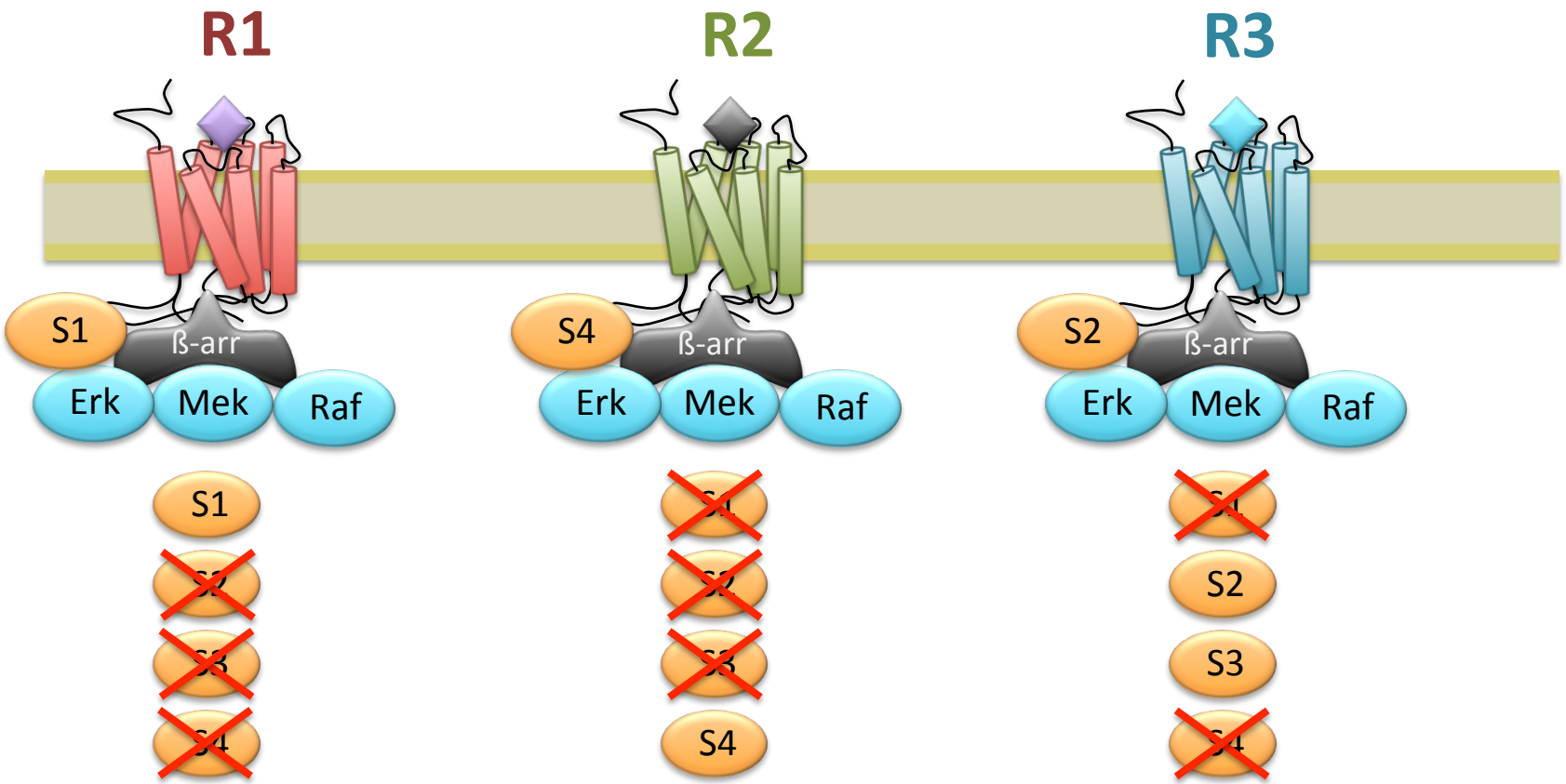
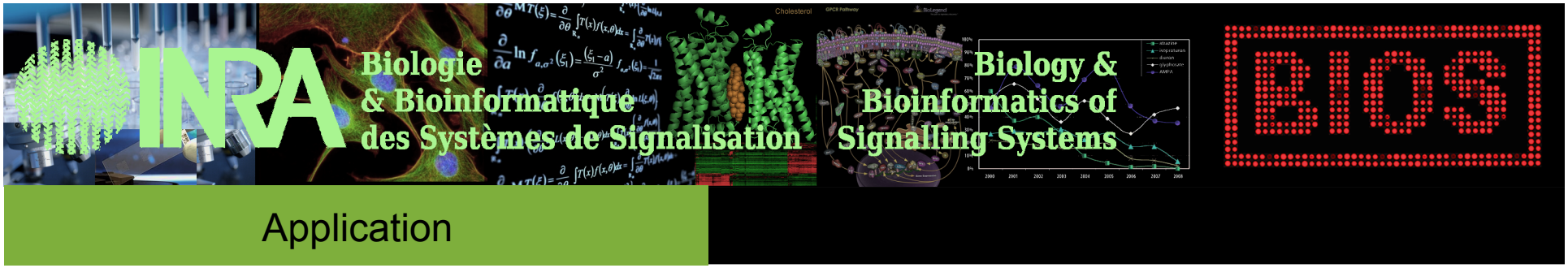


Modèle de l'assemblage par docking et validation expérimentale partielle => interaction directe entre le récepteur et le substrat de Erk.

Si c'est vrai, alors les substrats de Erk ß-arrestine-dépendants ne sont pas les mêmes pour des récepteurs différents.

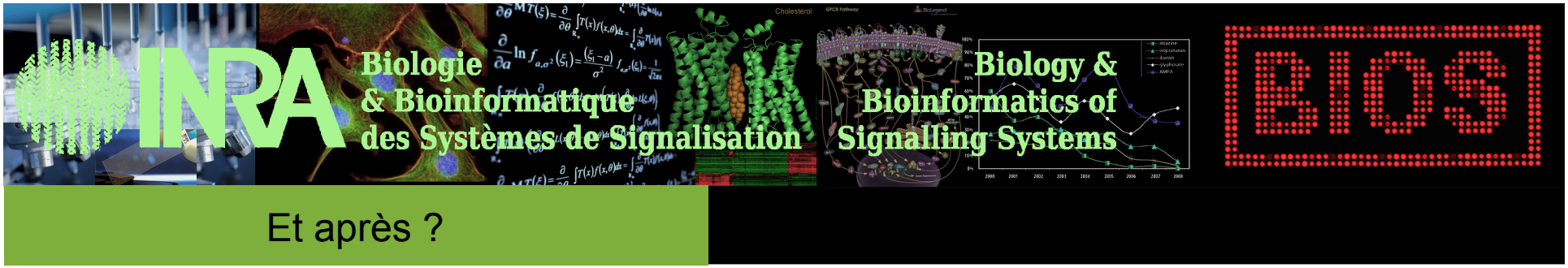




Application

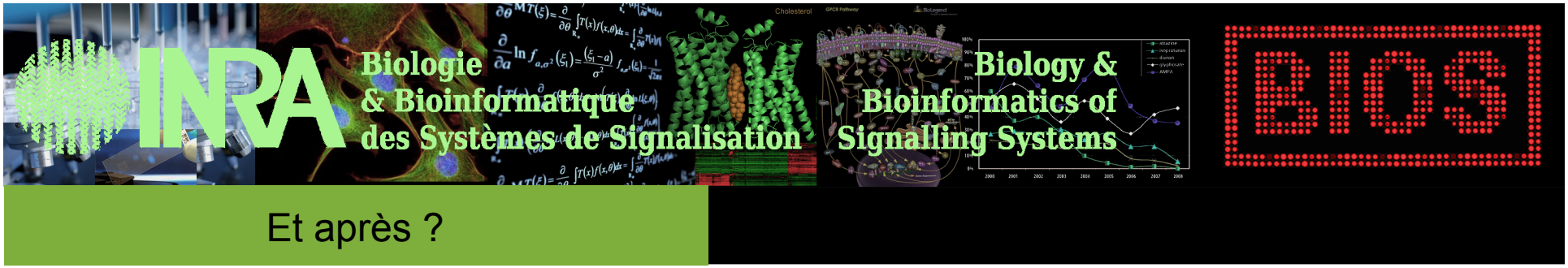
Démarche :

- Rechercher tous les articles contenant les mots-clefs « GPCR », « signaling », « arrestine » et « Erk ».
- Extraire les résultats expérimentaux.
- Reconstruire les réseaux de signalisation ß-arrestine et Erk-dépendants avec la méthode d'inférence.
- Faire une liste de tous les substrats de Erk trouvés et des différents récepteurs étudiés.
- Valider expérimentalement.



Et après ?

- On peut envisager de traiter des collections complètes de publications
  ⇒ possibilité de générer une base de données contenant les phrases significatives et donc de révolutionner la recherche bibliographique !



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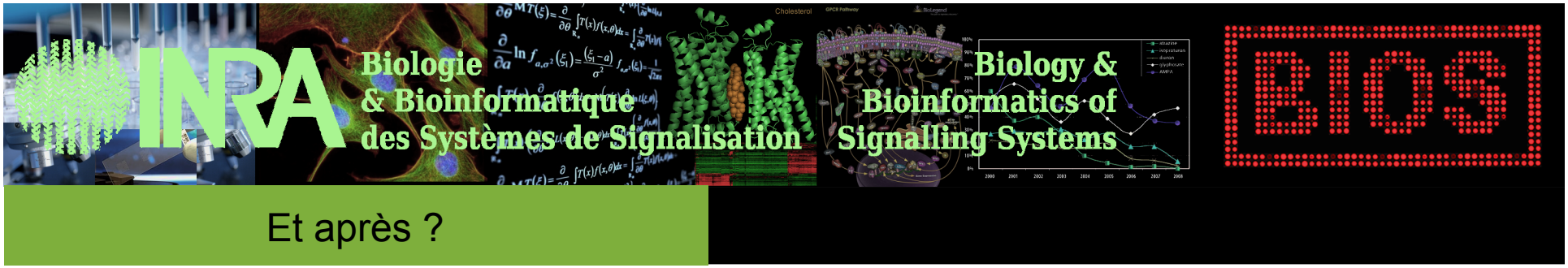
  ⇒ nécessité d'extraire également des informations plus générales dans l'article : récepteur étudié, type cellulaire, etc.



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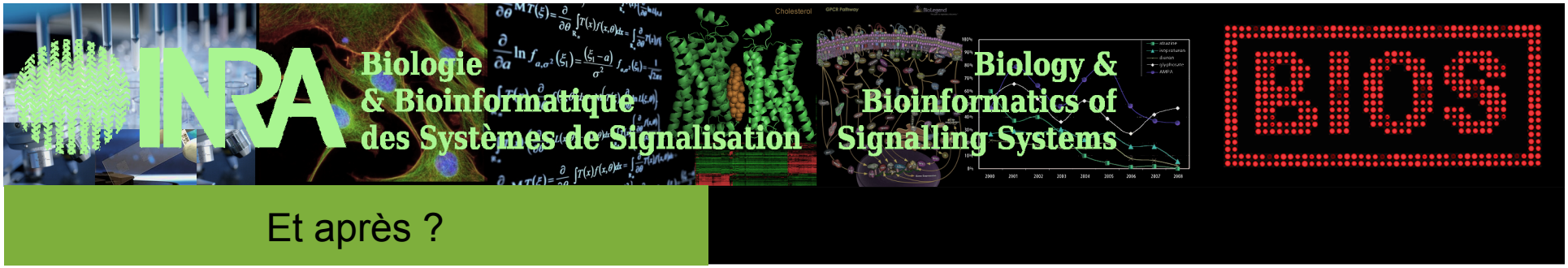
  ⇒ accès à d'autres revues. En particulier il sera difficile de se passer de JBC et Biochemistry



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- Etendre à d'autres domaines de la Biologie
  ⇒ étendre les dictionnaires